Remote Ischaemic Conditioning After Stroke 3 (RECAST-3): A multicentre randomised controlled trial

Final Version 5.0
07 May 2024

Short title: ***Remote ischaemic Conditioning After Stroke Trial - 3***

Acronym: *RECAST-3*

ISRCTN registry: ISRCTN63231313

IRAS Project ID: 277021 (England) 282606 (Scotland)

Trial Sponsor: University of Nottingham

Sponsor reference: 20011

Funding Source: NIHR Efficacy and Mechanism Evaluation

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2 SYNOPSIS

Title	Remote Ischaemic Conditioning After Stroke 3 (RECAST-3): A multicentre randomised controlled trial
Acronym	RECAST-3
Short title	*Remote ischaemic Conditioning After Stroke Trial 3*
Chief Investigator	Professor Tim England
Aim	To perform a multicentre randomised controlled trial assessing remote ischaemic conditioning (RIC) in patients with acute ischaemic stroke
Trial Configuration	Phase III prospective randomised (1:1) sham-controlled blinded-endpoint parallel-group multicentre trial.
Setting	Adults with acute ischaemic stroke presenting in Emergency Departments and Stroke Units in the UK.
Sample size estimate	Assuming alpha=0.05, power=90%, losses to follow up=5% and covariate adjustment reducing sample size by 20%, a sample size of 1300 will be needed to detect a treatment effect of OR 0.75 by shift analysis of mRS.
Number of participants	1300
Eligibility criteria	**Inclusion criteria:** Acute ischaemic stroke (≤24 hours post onset); spontaneous intracerebral haemorrhage ruled out on baseline clinical neuroimaging; NIHSS score 5-25 at randomisation; age ≥18 years. Mechanical thrombectomy sub-study only (selected sites) - received mechanical thrombectomy ≤24 hours post onset. **Exclusion criteria:** Pre-morbid dependency (modified Rankin Scale, mRS>3); spontaneous intracerebral haemorrhage; systolic blood pressure <80mmHg; haemorrhagic transformation of infarction PH2; pre-existing diagnosis of dementia; coma (GCS <8); malignancy; significant co-morbidity (life expectancy <6 months); BM <3.0mmol/L; known pregnancy; taking part in another interventional trial, unless co-enrolment has been approved by Chief Investigators and Sponsors; seizure on presentation unless brain imaging identifies evidence of significant brain ischaemia; significant tissue injury of the upper limbs, which in the opinion of the investigator, will be exacerbated by remote ischaemic conditioning; expected repatriation of the participant to another hospital not participating in RECAST-3 where RIC or sham cannot continue. Mechanical thrombectomy sub-study only (selected sites) – known contra-indication to administration of iv contrast (required for a CT Perfusion scan). This may include previous allergic reaction to contrast or considered high risk for contrast induced nephropathy (at the discretion of the investigator). This is not an exclusion for the main trial.
Description of interventions	**Intervention:** RIC group: 4 cycles of intermittent upper limb ischaemia - alternating 5 minutes inflation (+20 mmHg above systolic BP) followed by 5 minutes deflation of bilateral upper arm blood pressure cuffs.

	Comparator: Sham RIC. Bilateral upper arm blood pressure cuffs are inflated to 50 mmHg for 4 cycles (5 minutes inflation/5 minutes deflation). **Duration of treatment:** twice daily for 14 days (28 doses). 1 dose=4 inflation/deflation cycles.
Duration of study	Study Duration: Total trial duration 45 months. Participant Duration: 90±7 days.
Randomisation and blinding	Web based randomisation will occur immediately after consent, performed by the clinician taking consent. Randomisation will be 1:1 RIC: placebo, minimised on baseline prognostic factors. Follow-up measures will be performed by assessors blinded to treatment allocation
Outcome measures	**Primary Outcome**: Death or dependency at day 90 (modified Rankin Scale [mRS], ordinal shift analysis) recorded using central blinded telephone follow-up. **Secondary outcomes** (day 90): Cerebrovascular events; major adverse cardiac and cerebral events; acute kidney injury; disability; cognition; mood; frailty; quality of life; safety (death; neurological deterioration; intracranial haemorrhage, systemic embolism, serious adverse events) **Mechanisms**: Mechanical thrombectomy sub-study (Day 2-14 MRI; infarct growth and volume, oedema, perfusion).

3 ABBREVIATIONS

AE	Adverse Event
CI	Chief Investigator overall
CRF	Case Report Form
DAP	Data Analysis Plan
DMC	Data Monitoring Committee
GCP	Good Clinical Practice
ICF	Informed Consent Form
mRS	Modified Rankin scale
NIHSS	National Institutes of Health Stroke Scale
NHS	National Health Service
PI	Principal Investigator at a local centre
PIS	Participant Information Sheet
REC	Research Ethics Committee
R&D	Research and Development department
RIC	Remote ischaemic conditioning
SAE	Serious Adverse Event
TMG	Trial Management Group

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4 TRIAL BACKGROUND AND RATIONALE

INTRODUCTION

Stroke is the third leading cause of death worldwide and is devastating to both patients and carers. In the United Kingdom there are 100,000 strokes (85% ischaemic [IS], 10-15% haemorrhagic [mostly intracerebral haemorrhage, ICH]) and costs society ~£9billion/year.[1] There are only a few effective treatments for acute ischaemic stroke: aspirin is used widely but has a modest efficacy, [2] and alteplase, thrombectomy and hemi-craniectomy the converse.[3,4] Recent research has failed to demonstrate efficacy of novel drug treatments,[5] therefore, new approaches to reduce the burden of stroke on society are required. There is an urgent need to improve clinical outcomes in patients with ischaemic stroke. Reducing stroke severity and recurrence will improve functional dependency and the considerable social and financial burden to patients, carers and society.

Ischaemic reperfusion injury (IRI) occurs after an ischaemic stroke and clinically manifests as early recurrent stroke, symptomatic intracranial haemorrhage, swelling of the original infarct and neurological deterioration, which are common causes of worsening outcomes.[6-8] Remote ischaemic conditioning (RIC) uses repeated cycles of transient limb ischaemia and reperfusion and helps protect the brain from IRI. The mechanisms underlying RIC are not fully understood but have been attributed to release of neuro-humoral chemical messengers from the limb/s, resulting in immediate (first 2-3 hours) and late (24-72 hours) windows of protection from ongoing and delayed cerebral IRI.[9,10] In pre-clinical stroke, RIC reduces infarct volume and improves neurological scores through multi-modal mechanisms of action. For example, RIC improves blood brain barrier integrity and cerebral oedema through down-regulation of astrocytic aquaporin-4;[11] enhances cerebral blood flow through augmenting collateral pial and leptomeningeal arterial blood flow;[12,13] reduces infarct volume through anti-inflammatory,[14] anti-apoptotic [15] and anti-oxidant mechanisms,[16] ultimately protecting the mitochondrial permeability transition pore. Further, recent data from fifty healthy volunteers suggests a single dose of RIC induces a sustained increase in dynamic cerebral autoregulation.[17]

RIC is an attractive strategy since it bears minimal cost, should be safe and would be simple to administer by medics and allied health professionals. A typical protocol involves inflating a blood pressure cuff, applied to a patient's upper arm/s, to a level exceeding the systolic blood pressure for 5 minutes in order to induce ischaemia in the limb/s, followed by 5 minutes deflation to allow reperfusion. The cycles are repeated before (pre-conditioning), during (per-conditioning) or after (post-conditioning) the ischaemic event.

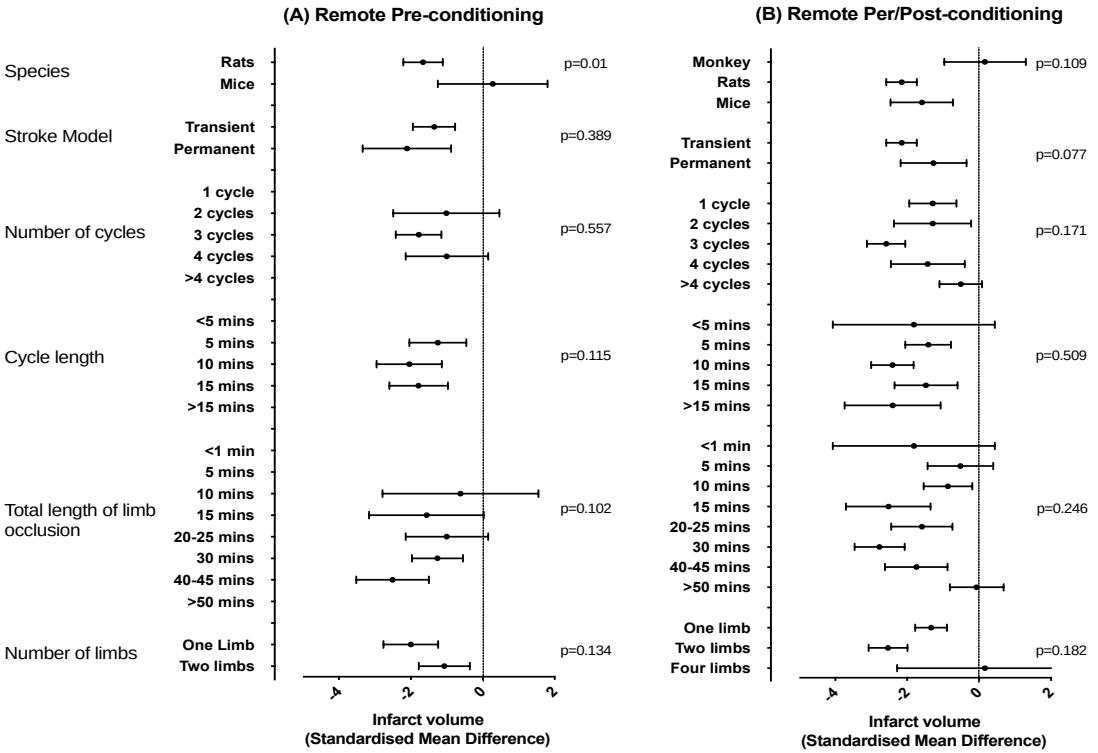
Following our pilot and feasibility studies, RECAST-1 & 2, we propose to perform a clinical phase III efficacy randomised controlled trial of RIC in acute stroke across multiple UK sites. The trial is also designed to address mechanisms of action including testing the effect of RIC on clinical markers of cerebral reperfusion injury, and an MRI sub-study evaluating infarct growth, volume and cerebral oedema.

Preclinical evidence

The mechanisms underlying RIC have been attributed to neuro-humoral pathways linking the pre-conditioned organ/tissue to the brain, resulting in attenuation of IRI (e.g. through enhanced collateral circulation and a decrease in cerebral oedema) and ischaemic tolerance mediated through a second window of protection.[9,10] Our pre-clinical meta-analysis in 1479 animals reveals that RIC significantly reduces infarct volume in both permanent (standardized mean difference [SMD] 1.59, $p<0.001$, Figure 1) and transient ischaemic models (SMD 1.93, $p<0.0001$) and improves neurological deficit (SMD -1.54, $p<0.0001$).[18] In Figure 1 we demonstrate the effect of different RIC administration parameters on infarct volume in rodent stroke models in both pre-conditioning and per/post-conditioning paradigms. In per/post-conditioned animals, 3 cycles of limb ischaemia and reperfusion was optimal (but not significantly different from 4 cycles), and a total length of limb ischaemia of 15-30 minutes led to the greatest degree of infarct volume reduction. There seemed to be better effect with using two limbs compared to one but this was not consistent with pre-conditioned stroke models where the reverse was seen. Importantly, a specific dose-

finding study in post-conditioned rats determined that 3 cycles of 5min/5min ischaemia/reperfusion (I/R) was more effective than 15sec/15sec & 8min/8min, and protection is seen if RIC is delivered up to 6 hours post onset.[19] Combining per- and post-conditioning may tackle both early and late phases of IRI;[20] alteplase combined with RIC has an additive effect;[21] and a single dose of RIC can have long-lasting protective effects for up to 6 days.[22]

Figure 1 Effect of remote ischemic per- and post-conditioning (RIPerC and RIPostC) compared to control on infarct volume, expressed as a standardised mean difference, by individual publication experiment



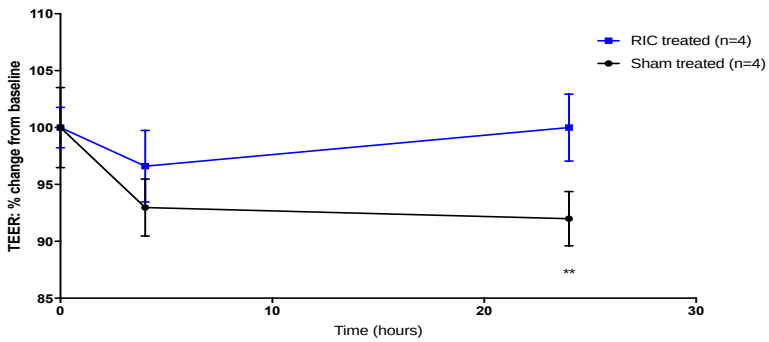
Clinical trials

Stroke

Hougaard 2014 administered RIC in the ambulance to suspected stroke (n=443). Penumbral salvage (the primary outcome) did not improve but there were more TIAs and less severe strokes on arrival to hospital in the per-conditioned group.[23] The trial was confounded by absent pre-randomisation measures, poor compliance and sub-threshold dosing (short ambulance transfer times). Therefore, delivering treatment on arrival to hospital is more practical whilst still achieving hyperacute administration.

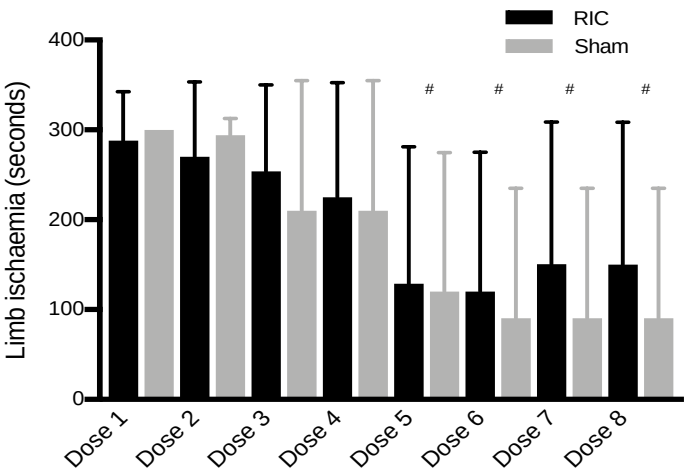
RECAST-1 (CI England, n=26) demonstrated excellent intervention tolerability using one dose of 4 cycles of upper limb ischaemia and reperfusion (5min/5min) performed with 24 hours of stroke, excluding those thrombolysed.[24] Although limited by a small sample size, there was a significant decrease in National Institutes for Health Stroke Scale (NIHSS) score in the RIC group at day 90 (median NIHSS 1 [0.5-5] versus 3 [2-9.5], p=0.04); RIC augmented neuroprotective proteins, plasma HSP27 and phosphorylated HSP27;[25] and there was a trend to fewer vascular events by day 90 (p=0·076, log-rank test). Further, in recently performed *ex vivo* experiments,[26] we used plasma acquired 4 days after RIC or sham

Figure 2. Effect of RIC treated plasma on *in vitro* BBB permeability: RECAST participants



from RECAST-1 participants and used the plasma to 'treat' an *in vitro* blood-brain-barrier (BBB) oxygen-glucose deprivation (OGD) model, mimicking stroke, which can be used to test trans-epithelial resistance (TEER) as a marker of BBB permeability.[27] 24 hours after OGD, there was a significant reduction in TEER (i.e. increased permeability) in the sham group (n=4) compared to RIC (n=4), (mean difference in change from baseline 14.75%, p<0.001, repeated measures ANOVA, Figure 2). IL-6 released from endothelial cells, neurons, pericytes and astrocytes in the model was significantly lower at 24 hours in the RIC group (225pg/mL versus 1061pg/mL, p=0.004, n=4/group). These data indicate that plasma obtained 4 days after a single 'dose' of RIC following ischaemic stroke displays neuroprotective properties, potentially through anti-inflammatory mechanisms.

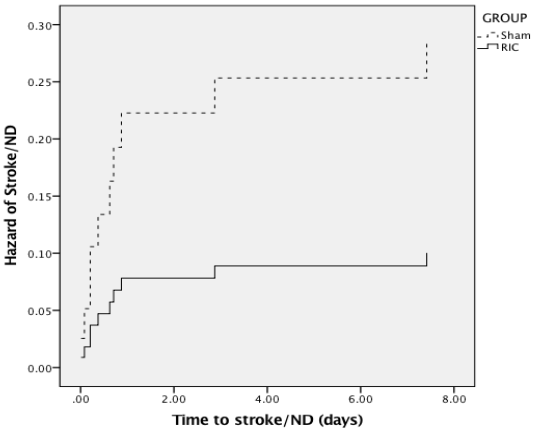
Figure 3. Mean (±SD) time of RIC adherence in RECAST-2. One dose = 4 cycles (5min/5min) of upper limb ischaemia/reperfusion



RECAST-2 (CI England, n=60, manuscript submitted) verified feasibility of RIC within 6 hours of acute ischaemic stroke (AIS);[28] RIC appeared safe using twice daily dosing for 4 days with a mean time to randomisation 4 hours 5 minutes; 55% received thrombolysis and there were no RIC related serious adverse events. RIC was well tolerated, adherence not differing between RIC and sham, but falling in both groups on day 3 (dose 5) to ~40% (# p<0.05, repeated measures ANOVA, Figure 3) due to early discharge or transfer. The sham was feasible since when asked at day 90 which intervention they received, 56 (93%) participants did not know, 2 (4%) were incorrect and 2 (4%) correct.

Biochemical signals of efficacy were evidenced by increased plasma biomarkers of brain injury (S100ß) in the placebo group (mean rise 111pg/ml (SD 302), p=0.041, repeated measures ANCOVA) not seen in the RIC group. S100ß is a recognised surrogate marker of infarct volume and functional outcome,[29] and in RECAST-2, S100ß correlated significantly with baseline stroke severity (NIHSS, r=0.561, p<0.001) and day 90 modified Rankin Scale (mRS; r=0.41, p=0.006). Further, in post-hoc analyses, there was a trend to reduction in recurrent cerebral events by day 90 in favour of RIC (adjusted hazard ratio [HR] 0.28, 3 vs 7 events, p=0.08, cox regression, adjusted for age, sex and baseline stroke severity, Figure 4). 82% of recurrent events (including recurrent/extension of ischaemic stroke, haemorrhagic transformation of infarction and neurological deterioration) occurred in the first 48 hours. There were no losses to follow-up.

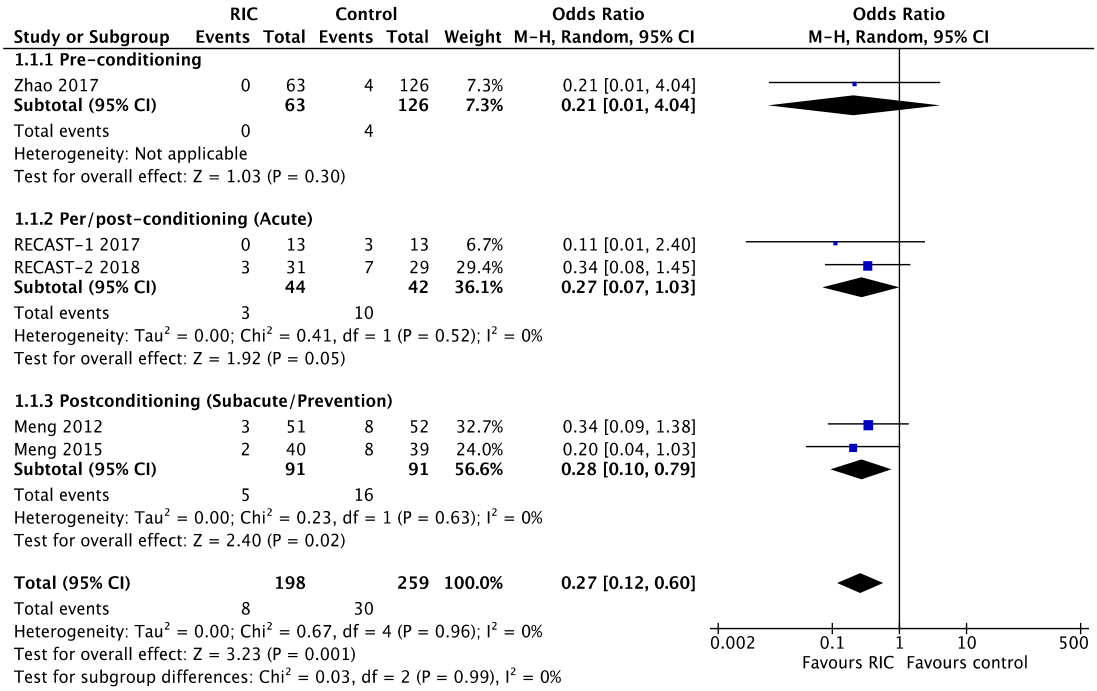
Figure 4. RECAST-2: risk of recurrent stroke and neurological deterioration (fatal and non-fatal) by RIC or sham



A recent proof-of-concept trial utilised remote ischaemic pre-conditioning 2 weeks prior to carotid stenting in a Chinese cohort with severe carotid stenosis (n=189);[30] RIC led to significantly fewer new DWI lesions on brain MRI in the RIC group compared to sham and control. Further, post-conditioning, using regular RIC may be effective in reducing recurrent ischaemic stroke. In two small RCTs, participants with intracranial arterial stenosis received twice daily bilateral upper limb

RIC for 300 consecutive days, starting approximately 10 days after their index event;[31,32] in association with improvements in cerebral blood flow, the treatment groups experienced fewer recurrent strokes. In updating the recent Cochrane Review [33] in RIC for preventing and treating ischaemic stroke (with RECAST-1&2), and organising groups into pre- per- and post-conditioning trials, RIC significantly reduces the composite outcome of recurrent vascular events, an odds ratio, OR 0.27 (95% CI 0.12-0.60, p=0.001), Figure 5.[28] This is consistent with secondary analyses in the cardiac literature (RIC and acute myocardial infarction, MI) where recurrent cardiovascular and cerebrovascular events were reduced by half.[34] It is not intuitive that brief periods of RIC can lead to protection from vascular events at much later time points (and repeated doses may be required) but the finding deserves further exploration in clinical trials.

Figure 5. Recurrent vascular events (non-fatal and fatal stroke, non-fatal and fatal MI) in RCTs of RIC in stroke populations



Ongoing and recent stroke RIC studies

We have performed a review of the current literature and screened for ongoing international trials regarding RIC and acute stroke using the international clinical trials platform registry (http://apps.who.int/trialsearch/). Paramedic initiated RIC RCTs RESIST (NCT03481777) and REMOTE-CAT (NCT03375762) are hampered by heterogeneity (IS, haemorrhagic stroke, mimics) and accuracy in measuring baseline stroke severity in the ambulance (a vital prognostic confounder). RESCUE-BRAIN (NCT02189928, France) selected 200 participants using MRI, applying RIC to the leg. REPOST (Netherlands Trial Register, NTR6880) used twice daily upper limb RIC for 4 days in AIS, started within 12 hours of onset. Similarly, the RICAMIS trial (NCT03740971, n=1893, China) performed upper limb **RIC within 48 hours** of ischaemic stroke for 10-14 days, excluding those undergoing reperfusion therapy, and demonstrated an improvement in functional outcome (mRS) by 90 days[35]. There are no other large-scale UK trials of RIC in AIS. Several other small Chinese studies are registered: AIS with thrombolysis (rtPA-RIC n=60, 'tripcais' n=120) and MT (REVISE-2, n=180). In subacute IS, a Chinese RCT sICAS (NCT02534545) used RIC daily for 300 days in symptomatic intracranial arterial stenosis. Whilst the trial did not show a significant reduction in recurrent stroke (primary outcome), the composite secondary endpoint of reduction in stroke and MI was significantly better in the treatment group[36]. RIC trials in post-stroke fatigue (NCT03794947) and motor recovery in chronic stroke (NCT03095755) are registered but not directly relevant to this trial.

The stroke trials delivering RIC in the first 24 hours after stroke (per-conditioning) published to date are summarised in the table below. All are small trials, testing proof-of-concept, tolerability and feasibility. None were large enough or designed to test the efficacy of RIC on clinical outcomes, hence the need for larger phase III trials.

Author, Year of Study, Country	Patient population	N	Location of RIC	Number of Cycles; Inflation/Deflation	Time of RIC	Effect on Neurological outcome
Hougaard et al, 2013, Denmark	AIS with IVT (n=443)	RIC = 73 Sham = 64	Upper Limb; 200mmHg or 25mmHG above	4 cycles; 5min x 5min	Pre-hospital (once)	No significant difference in mRS at 90days
England et al 2017, UK	AIS without IVT (n=26)	RIC = 13 Sham = 13	Upper Limb; 20mmHG above SBP in RIC, 30mmHg in Sham	4 cycles; 5min x 5min	Once after 6hrs but within 24 hours of onset	Improved NIHSS scores at 90days. No significant difference in mRS at 90 days
England et al 2019, UK	AIS with/without IVT (n=60)	RIC = 31 Sham = 29	Upper Limb; 20mmHG above SBP in RIC, 30mmHg in Sham	4 cycles; 5min x 5min	Once within 6hrs of onset then up to twice daily for 4 days:	No significant difference in mRS, BI at 90days & NIHSS at 4 days
Che et al, 2019 China	AIS with IVT (n=30)	RIC = 15 Sham = 15	Both Upper Limbs; Auto device; 200mmHg RIC, 0mmHg Sham	5 cycles; 5min x 5min	Once within 2 hours of IVT (IVT within 4.5hrs of onset) then twice daily for 6 days	Significant reduction in NIHSS on day 30 in RIC. No significant difference in 90-day mRS, BI & NIHSS
He et al, 2020, China	AIS with IVT only (n=49)	RIC = 24 Sham = 25	Upper limb; 200mmHg RIC, 60mmHg Sham	4 cycles, 5min x 5min	Twice within 6-24hrs of IVT (first given 6hrs after IVT)	No significant difference in mRS at 90days, NIHSS at 1,7 & 30 days
An et al, 2020, China	AIS with IVT only (n=68)	RIC = 34 (2 excluded) Sham = 34	Both Upper Limbs; Auto device; 180mmHg RIC, 0mmHg Sham	5 cycles, 5min x 3min	Twice daily during hospital stay 8-14days (first within 3hrs of IVT)	Improved mRS 0-1, mRS 0-2 and NIHSS scores at 90days.
Pico et al, 2020, France	AIS with/without IVT (n=188)	RIC = 93 Sham = 95	Lower limb; Auto device; 110mmHg above SBP in RIC, 0mmHg in Sham	4 cycles; 5min x 5min	Once within 6 hours of onset	No significant difference in mRS at 90days

Since the initial conception of RECAST-3, there have been delays in trial set-up with respect to provision of an RIC Device. In the meantime, the RESIST trial presented its results to the European Stroke Organisation Conference in May 2023.

The Danish RESIST investigators enrolled 1500 participants within 4 hours of onset in a pre-hospital setting, of which 737 were ischaemic stroke, 165 ICH (n=165) and the remainder stroke TIA (10.5%) or mimic. Randomised 1:1 to single limb RIC or sham, 80% received 7 days of twice daily treatment (20% 1 day of treatment); no differences were found between treatment and control groups in the primary outcome, shift in modified Rankin scale.

We now have one positive phase III trial from China (RICAMIS) and a neutral trial (RESIST). Outlined in the table below are the key differences between RICAMIS, RESIST and RECAST-3 in its original design.

In short, the positive RICAMIS trial had a **longer treatment** duration and **used two limbs** for RIC reflecting a higher RIC dose.

The RECAST-3 treatment protocol has been modified (right of the table) in response to the results of both RICAMIS and RESIST. These changes reflect an increased RIC dose through the following modifications:

1) twice daily RIC for 14 days (or until discharge, whichever comes sooner). The aim is for 14 days/28 doses of treatment, accepting that 2 days missed out of 7 is acceptable by protocol as some centres will not be able to deliver at the weekends (see section 7.1).

2) RIC on both upper limbs - the device from AneticAid is able to deliver this without modification.

Other changes:

3) Time of intervention - randomise within 24 hours (not 6 hours)

4) Refine range of stroke severity included (NIHSS score 5-25)

5) Remove the need for mandating a second CT head scan on day 2 in all patients. RIC seems safe in the preceding large trials and we will measure *clinical* cerebrovascular for safety.

6) RIC inflation pressure +20 mmHg above systolic (not +25 mmHg)

7) Sham pressure 50 mmHg (not 20 mmHg)

	RICAMIS	RESIST	RECAST-3 Pre Amendment	RECAST-3 Modified
Country	China n=1776 (913/863)	Denmark Sham 466 RIC 434	UK N=1300 planned	Unchanged
Population	Moderate ischaemic stroke NIHSS 6-16	Pre-hospital IS 83% ICH 17% [mean NIHSS on admission 5]	NIHSS ≥4 IS	NIHSS 5-25
Includes IVT?	No	Yes (66%)	Yes	Unchanged
Includes MT?	No	Yes (19%)	Yes	Unchanged
Time of intervention	Within 48 hours of onset	Within 6 hours of onset	Within 6 hours	Within 24 hours
RIC Intervention	Bilateral upper limb 200 mmHg	Single upper limb 200mmHg	Single upper limb +25 mmHg above systolic	Bilateral upper limb +20 mmHg above systolic
Dose	10-14 days bd	up to 7 days bd in 80%	2 days bd	10-14 days
Sham?	No sham	Sham pressure 20mmHg	Sham pressure 20 mmHg	Sham pressure 50 mmHg
Result	Positive (mRS shift) acOR 1.37 (95% CI, 1.16-1.63 P<0.001)	Neutral acOR 1.05 (0.83-1.33,p=0.67)	-	-

Cardiac Trials

Cardiac preconditioning: Two large trials in remote ischemic preconditioning in patients undergoing coronary-artery bypass grafts (CABG) did not reduce major adverse cardiac and cerebral events (MACCE) [37,38], reasons for this included the potential interaction with the anaesthetic agent Propofol [39], which diminishes the effects of RIC. In the setting of elective percutaneous coronary intervention (PCI) (low to moderate risk patients), the results are mixed and performed in relatively small studies.

Cardiac per-conditioning: Data from phase II trials in RIC for myocardial infarction are encouraging, demonstrating reductions in myocardial infarct size, cardiac biomarkers and myocardial oedema (e.g. [40,41]). A systematic review of these studies suggests the significant reduction in myocardial damage may not be clinically meaningful [42]. However, a more recent larger single centre trial not included in the analysis randomised 516 patients with acute ST-elevation MI (STEMI) to RIC or control [43]; composite primary outcome of cardiac mortality and hospitalisation for heart failure was significantly reduced in favour of RIC: HR 0.35 (95%CI 0.15-0.78). In addition, follow-up of the CONDI trial of RIC in STEMI patients showed less MACCE at median follow-up of 3.8 years (all-cause mortality, myocardial infarction, readmission for heart failure, and ischaemic stroke/transient ischaemic attack with RIC (13.5%) when compared to control (25.6%) [34]. Another recent small trial used daily RIC continued for 4 weeks after acute MI in 73 patients [44]. Left ventricular function did not improve but treatment was started as late as day 3 when chances of rescuing salvageable tissue would have been small.

The phase III 5400 STEMI patient CONDI2/ERIC-PPCI (Effect of Remote Ischaemic Conditioning on Clinical Outcomes in STEMI Patients Undergoing PCI) trial [45] showed that RIC had no effect in improving cardiac clinical outcomes at 12 months when administered in the pre-hospital setting in patients with suspected STEMI and who were eligible for PPCI. However, there are several key differences between CONDI-2/ERIC-PPCI and RECAST-3 in both the populations studied and the trial design:

(1) Population: Patients with STEMI are pre-conditioned through effective cardiac treatments that are not effective nor used routinely in hyperacute stroke, namely, opiates (50% in CONDI-2/ERIC-PPCI), heparin (85%), ADP inhibitors (clopidogrel 26%, ticagrelor 69%, prasurgrel 4.5%), nitrates (78%), glycoprotein IIb/IIIa inhibitors (19%), and bilvalirudin (22%). A number of these treatments are known to interact with the effects of RIC, in particular nitrates [46] and drugs modulating opioid receptors [47]. A further potential reason for the neutral results in CONDI-2/ERIC-PPCI is that 95% of recruits were of lower risk, Killip class I at randomisation (no heart failure). Patients with STEMI are so well treated in the hyperacute phase that it has diminished ischemia-reperfusion as a target for protection. In ischaemic stroke, however, there are no proven adjunctive therapies to accompany reperfusion strategies thrombolysis and thrombectomy, even aspirin is avoided in the first 24 hours after thrombolysis. Hence, treatment of reperfusion injury remains a key target in improving outcomes post stroke.

(2) Trial design: In addition to treating a different organ, other key differences include (i) the use of repeated RIC dosing over 2 days as used in RECAST-2 [48] (compared to a single ‘dose’ in CONDI-2/ERIC-PPCI) and (ii) the timing of the intervention, which will be applied on arrival to hospital in RECAST-3 rather than in the ambulance as in CONDI-2 (administration at reperfusion versus pre-perfusion).

Although there are clear similarities in both cardiac and stroke populations, the effects of RIC in acute ischaemic stroke needs to be considered on its own merit. Overall, there are significant and sufficient differences in the populations studied and trial design to warrant phase III trials in acute stroke.

4.1 DETAILS OF INVESTIGATIONAL MEDICAL DEVICE

Device Description

Model: AT4 Electronic Tourniquet System
Manufacturer: Medical Device Management Ltd
Distributed by: Anetic Aid Ltd

The tourniquet has been classified as a 'Class IIa' medical device in accordance with the European Medical Device Directive 93/42EEC as amended by 2007/47.

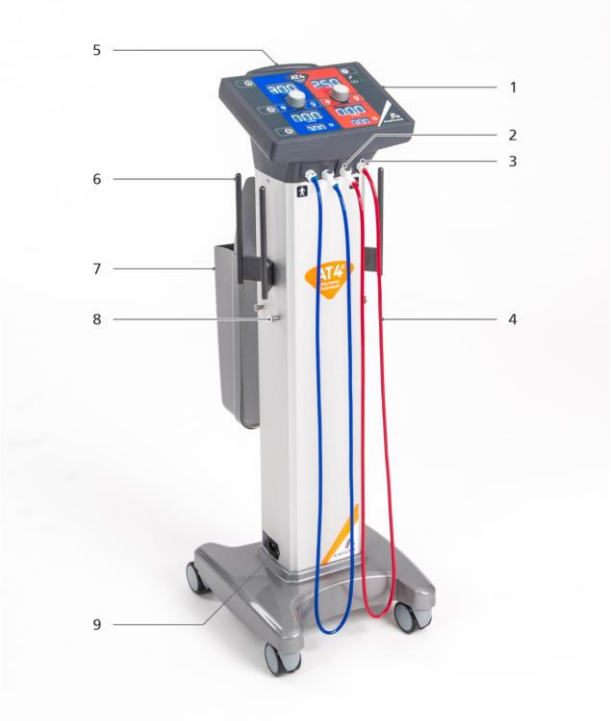
The AT4 Electronic Tourniquet System has a CE mark (631139) for inducing limb ischaemia for prolonged periods of time. The device inflates for a set (modifiable) period of time at a fixed (modifiable) pressure, as determined by the user.

The CE mark for the device covers an intended use of "inflation of pneumatic tourniquet cuffs within the range of pressure up to 600 mmHg to occlude the blood flow [and to obtain a bloodless field during limb surgery or amputation]. Both manufacturer and trial Sponsor are in agreement that this covers the intended use in RECAST-3 and as such does not fall into the category of an investigational medical device when used in this trial. A letter of no objection from the MHRA is therefore not required for this trial

The same device will be used to deliver both RIC and sham protocols.
- The RIC protocol will consist of 4 cycles of 5 minutes bilateral cuff inflation to +20 mmHg above systolic BP (inducing temporary limb ischaemia) and deflation (limb reperfusion period) over 40 minutes.
- The sham protocol will consist of 4 cycles of 5 minutes bilateral cuff inflation to 50 mmHg and deflation over 40 minutes.

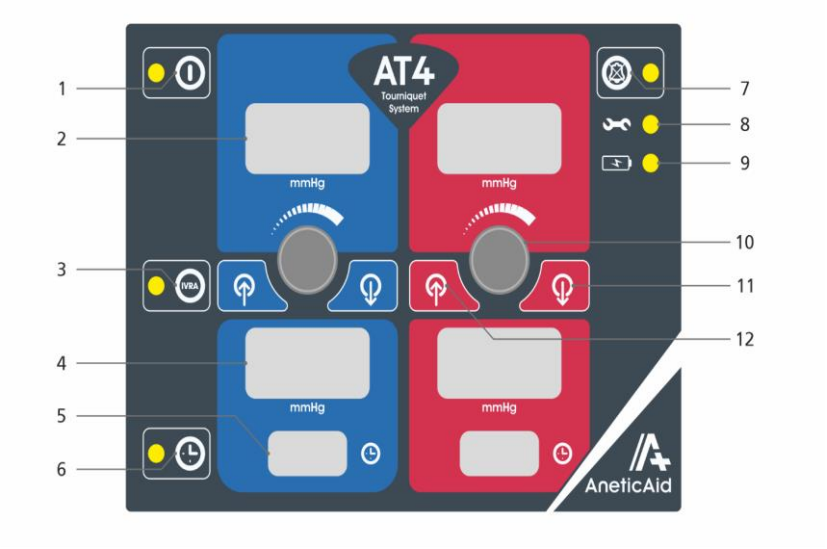
The investigator will set the inflation pressure and attend to the device every 5 minutes to elicit the periods of inflation and deflation.

Product Functions:



1. Control Panel
2. Cuff Supply Hose Storage Connectors
3. Cuff Supply Hose Connectors
4. Cuff Supply Hose
5. Pulling Handle
6. Cuff Hooks
7. Storage Facility
8. Additional Storage Facility Locating Pins
9. IEC Socket

Product Controls and Operation



1. ON/OFF
2. Set Pressure display
3. IVRA (Intravenous Regional Anaesthesia) **Not applicable for RECAST-3**
4. Applied Pressure Display
5. Elapsed Time H:MM
6. Reminder control **Not applicable for RECAST-3**
7. Audible alarm, pause and indicator
8. Maintenance indicator
9. Battery level indicator
10. Pressure controller
11. Deflate button
12. Inflate button

Symbols used on the AT4 tourniquet control panel:

Symbol:	Title:	Description:
	ON / OFF	Press to turn ON, Green indicator. To turn OFF press and hold until pressure displays are blank.
	AUDIBLE ALARM PAUSE	Press once to pause audible alarms for 3 minutes; Amber Indicator (except Low Battery).
	AUDIBLE ALARM OFF	Press second time to cancel audible alarms (except Low Battery). Red Flashing Indicator.
	INFLATE PRESSURE CONTROL	Turn to set inflation pressure blue channel. Turn to set inflation pressure red channel.
mmHg mmHg	PRESSURE SET OR APPLIED	Indicates the set and applied pressures.
	INFLATE	Inflates blue and red channels respectively.
	DEFLATE	Deflates blue and red channels respectively.
	TIME	Indicates the timer display for blue and red channels respectively.
	BATTERY LEVEL	Green: represents acceptable battery level Amber: connect to mains as soon as soon as practical. Green/Amber: Charging. Red: connect to mains immediately.
	MAINTENANCE INDICATOR	Amber: Service required. Red: Stop using and request immediate service.

Description of storage and installation of the device

Storage

- Operating conditions
    - Temperature: 15°C to 35°C
    - Humidity: 20% to 80% non-condensing
    - Height above sea level to be less than 2000m
- Movement and storage between use
    - Temperature: 5°C to 40°C
    - Humidity: ≤80% non-condensing
    - Atmospheric pressure 50kPa – 113kPa
    - Floor to be level to within 10° of horizontal when being moved
    - Not suitable for negotiating steps or thresholds
- Handling
    - The AT4 should not be pushed as equipment is more stable and controllable when pulled by the handle.
    - The castors are intended for repositioning the AT4 within the operating room environment or on other smooth level surfaces and slopes up to 10°. They are not intended for negotiating steps, thresholds or other obstacles such as cables or hoses.
    - If required to be lifted up a step or over a threshold the AT4 should be lifted by the cuff hooks on the side of the unit. Do not lift the AT4 by the control panel as this may result in damage.

Fitting the Fuse and Charging the Battery:
For safety, the AT4 is shipped without the batteries being operational; the fuse is removed during final inspection. The unit will have been supplied with a T3.15A fuse in a clear plastic bag with the operating instructions; this needs to be fitted during commissioning.

Lay the AT4 on its back, and fit the fuse in the fuse holder indicated by the fuse symbol on the underside of the AT4.

On receipt, or after periods of storage, the AT4 must be connected to the mains electricity supply with the cable provided for 24 hours to allow the battery to be charged.

When fully charged the AT4 may be disconnected from the mains, and operated from the battery, avoiding the requirements for mains cable in the clinical area. The AT4 may also be operated while connected to the mains if the battery is low.

When not in use it is recommended that the AT4 be left connected to the mains to ensure that the battery is fully charged and ready for use.

Connecting, and storing, the cuff hoses:
The AT4 will have been supplied with Red and Blue cuff hoses. These should be connected to the connections on the front of the AT4 and below the appropriate red or blue segment of the front panel. There are two connections to pressurise the cuffs, and two connections which are for stowage of the cuff end of the hoses when not in use.

Equipment check:
Ensure that the battery has been charged and the battery indicator is green; if not it must be used connected to the mains supply.

The red and blue cuff hoses should be connected to the connectors in the front of the AT4 ready for use below the appropriate red or blue segment of the front panel. There are two connections to pressurise the cuffs and two which are for stowage of the cuff end of the hoses when not in use.

Select the appropriate size and type of cuff(s) and apply to the patient's upper arms. The correct size and shape of cuff will allow cessation of blood flow at lower pressures and reduce the risk of harm to the tissue.

Before use, ensure all device functions operate correctly. Also visually inspect the device for any loose or damaged parts. If the devices performance changes from that specified or required, the device should be taken out of service immediately. Ensure that O rings on cuffs and associated hoses are in good condition before use.

Intervention delivery:
Before each 'dose' of the RIC or sham intervention, the investigator will inspect the participant's arms and skin condition, and make a note of any skin changes or damage.

Switching on:
- The AT4 is switched on by depressing the ON button. The LED will be lit green.
- If the internal air reservoir requires it, the internal compressor may be heard for a brief period.

Connecting the tourniquets cuffs
- After applying the tourniquet cuffs to both upper arms:
- At the end of the cuff supply hose connected to the intended channel of use, depress the metal connector clip before fully inserting the tourniquet cuff connector.
- Ensure the connector is fully inserted and secure.

Elapsed inflation time
- It will be necessary to use a standalone timer to ensure that the cuffs are inflated and deflated at 5-minute intervals for the duration of the intervention (4 cycles: 40 minutes total).

Pressure selection
- A blood pressure measurement must be taken immediately prior to pressure selection.
- Set the required pressure on both channels by rotating the control clockwise to increase and anticlockwise to decrease.
- The selected pressure in mmHg is displayed in the window above the rotary control.
- The following values should be used according to whether the participant has been randomised to receive RIC or Sham:

  RIC: +20 mmHg above systolic blood pressure.

  Sham: 50 mmHg.

  NOTE: At 450mmHg and above, an audible beep will be heard to draw the user's attention to the pressure selection.

  Application of a tourniquet cuff at excessive pressures can result in tissue necrosis.

Inflating a tourniquet cuff
- To inflate the cuffs, depress the inflate buttons on both channels in turn.
- The applied pressure displays will now illuminate and display the applied pressure.
- The cuffs should remain inflated for a period of 5-minutes before being deflated.

  NOTE: Once inflated, reducing the pressure below 250mmHg an audible beep will be heard to draw the user's attention to the pressure selection.

Deflating a tourniquet cuff
- To deflate the cuffs, depress the deflate buttons on both channels in turn.
- A single push initiates a slow deflate a second depression initiates a fast deflate.
- During deflation the screen will flash.
- The cuffs should remain deflated for a period of 5-minutes prior to reinflation (until the 4th cycle has been completed).

Post intervention
- When the intervention is finished, press the off button to turn the AT4 off as this will conserve battery life.

When not in use it is recommended that the AT4 be left connected to the mains to ensure that the battery is fully charged and ready for use.

Please refer to the Product Training Guidance document for the AT4 Electronic Tourniquet for further guidance on determining the battery status, identifying alarms and warning indicators, and maintenance.

Packaging and labelling
Anetic Aid Ltd will supply the devices to the UK Coordinating Centre in Nottingham and the trial sites.
Device label:
The following label will be added to the devices to demonstrate that the devices are to be used for research use only.

Control Devices
No separate control devices will be provided, as detailed in section 4.1.1. The same device will be used to deliver both RIC and sham protocols, requiring the investigator to set the inflation pressure manually depending on the randomisation result.

Storage, supply and return
The trial management team will be responsible for supplying the devices, which have been supplied by Anetic Aid Ltd, with each centre receiving one device. When not in use, the device should be held in a secure location, and in accordance with guidelines outlined in the Product Training Guidance document.

Known Device Effects
The expected effects are explained extensively in the background section. Trials of RIC in stroke and other conditions have not reported any significant concerns on the safety of RIC with respect to SAEs (specifically, local tissue damage).[23,24,40] There were no reported complications subsequent to thrombolysis in participants in RECAST-2. Skin petechiae caused by cuff inflation are the only expected non-serious adverse event in response to the RIC stimulus. Further, there were no safety concerns in the recently reported CONDI2/ERIC-PPCI, RICAMIS and RESIST trials.[35,45,49]

Unexpected adverse events will be reported to Nottingham Stroke Trials Unit. Confirmed unexpected SAEs will be notified to the Sponsor, Research Ethics Committee and Data Monitoring Committee.

Accountability for devices

The investigator, or an approved representative, will ensure that all investigational devices are stored in a secure area, under recommended storage conditions and in accordance with applicable regulatory requirements. All devices will be accounted for by the investigator using device accountability forms.

5 TRIAL OBJECTIVES AND PURPOSE

5.1 PURPOSE

To perform a multicentre randomised controlled trial assessing remote ischaemic conditioning (RIC) in patients with acute ischaemic stroke (AIS)

Hypothesis:
Remote ischaemic perconditioning (RIC) is safe and improves functional outcome in patients presenting with acute stroke.

5.2 PRIMARY OBJECTIVE

Primary research question: Does RIC improve functional outcome (ordinal shift in modified Rankin Scale, mRS) at day 90 in patients with acute ischaemic stroke?

5.3 SECONDARY OBJECTIVES

Secondary research questions
1. Does RIC reduce early and recurrent cerebrovascular events by day 90 in patients with AIS?
2. Does RIC impact on other clinical outcomes at 3 months: major adverse cardiac and cerebral events (MACCE); acute kidney injury (AKI); cognition; mood; frailty; and quality of life?
3. Is RIC safe when applied in patients with hyperacute stroke?
4. Does RIC reduce brain tissue injury associated with reperfusion? (MT sub study)

6 TRIAL DESIGN

6.1 TRIAL CONFIGURATION

Design: Prospective randomised sham-controlled blinded-endpoint parallel-group multicentre trial of RIC versus control. 1,300 patients with acute ischaemic stroke will be randomised 1:1 across 60 UK based NHS Trusts.

Endpoints will comprise of comparisons between RIC and sham:

Primary outcome
Functional outcome at day 90 (mRS, ordinal shift analysis) conducted by central telephone follow-up blinded to treatment allocation.[50] (Appendix A)

Justification of primary outcome: If RIC reduces cerebral ischaemic reperfusion injury and recurrence of cerebrovascular events, this should positively impact on functional outcome by day 90. The mRS is the outcome measure of choice in large acute stroke trials [39] and central telephone follow assessment ensures blinding of treatment allocation as we have previously used.[8, 38, 40] The timing of the primary outcome at 90 days is standard in most acute stroke trials and reflects that the primary outcome should measure the effect of treatment and a period thereafter to ensure that treatment effects are not lost.

Secondary outcomes
Clinical (day 90):
Cerebrovascular events by day 90;† mRS (binary);[50] major adverse cardiac and cerebrovascular events (MACCE: cardiovascular death, MI, all stroke); acute kidney injury, AKI;[51] disability (Barthel Index, [BI], dysphagia [functional oral intake scale[52]]); cognition (TICS-M); mood (Zung Depression Scale); Frailty (Clinical Frailty Scale, CFS);[53] Quality of Life (EQ-5D-5L); home-time;[7,8] recorded with mRS via telephone. (See Appendix B-H)

Compliance: recorded by investigators**.**

Justification of secondary outcomes: Differences in cerebrovascular events (defined below)

should reflect the expected mechanisms by which RIC is intended to improve outcome by protecting against ischaemic reperfusion injury. Measures of disability, cognition, mood and quality of life may all improve as a consequence of improved functional outcome and are accepted secondary outcome measures. They are convenient to measure over the telephone and recommended to measure by the European Stroke Organisation.[8, 44]. Frailty predicts inpatient morbidity and mortality but its link to functional outcomes is under researched;[45] frailty is a clinical parameter that can predict clinical outcomes independently of disability or comorbidity. Pre-morbid frailty is a potential confounder whilst frailty at follow-up is an important outcome measure.[42, 46] Health economics: As this is a pragmatic study, we will not perform a fully integrated economic analysis. However, differences in quality of life as measured by a cardinal utility instrument will be collected at day 90. The EQ-5D is a brief and simple instrument taking a few minutes to complete hence keeping participant burden to a minimum. In addition to EQ-5D we will collect data on length of hospital stay, home-time, discharge destination and return to work, all of which impact on cost effectiveness. However, as RIC is a simple, inexpensive treatment it is hypothesised that any positive effect on outcome will be cost-effective.

Safety endpoints

Safety (day 2, end of treatment & day 90)**:** death; recurrent IS, intracranial haemorrhage, symptomatic swelling of the original infarct;[6] neurological deterioration; transient ischaemic attack (TIA); systemic embolism, neurovascular limb compromise.

SAEs after the RIC/sham treatment period (i.e. >20 days after randomisation) will not be collected; thereafter, only fatal SAEs and outcomes will be recorded and blindly adjudicated until day 90.

Mechanistic studies

Sub-study: Mechanical thrombectomy, MT (n=80).

Participants in this mechanical thrombectomy sub-study will all receive a CT Perfusion (CTP) brain scan which may be standard of care or an additional exposure, depending on the standard practice at the participating centre, prior to the trial intervention. A standard of care MRI brain scan will be performed at day 2-14 assessing the pleiotropic effects of RIC:

(i) Infarct volume - Day 2-14 FLAIR volume, which correlates significantly with final infarct volume (correlation coefficient 0.93);[54]
(ii) Infarct growth: Day 2-14 MR FLAIR stroke volume - Day 1 CTP infarct core.[55,56]
(iii) Cerebral oedema: using region of interest analysis, we will partition swelling from infarct volume,[57] both are independently associated with a poor outcome;
(iv) Cerebral perfusion: based on arterial spin labelling (ASL, as available), to non-invasively quantify reperfusion status post-thrombectomy, correlates with early neurological outcome;[58]
(v) Haemorrhagic transformation of infarction, HTI (T2*-weighted imaging or SWI)

Initially, this substudy will only be performed at University College London (UCL) and Nottingham University Hospitals NHS Trust. If other centres express a wish to take part, this will be considered depending on MRI and CTP availability and the development/standardisation of the protocols.

When offering the substudy to potential participants, the risks of CTP need to be considered and inclusion will be at the discretion of the investigator. Risks include those of ionising radiation (see section 7.3) and the risks of administering intravenous contrast agents. Risk of contrast induced nephropathy is rare (<1%) but is increased in those with pre-existing renal disease, dehydration and if taking metformin. Steps to reduce these risks will be aligned with the centre's usual clinical practice when administering contrast and may include avoiding metformin and hydration.

6.2 RANDOMISATION AND BLINDING

All participants eligible for inclusion and for whom consent has been obtained will be randomised centrally using a secure internet site in real-time. Randomisation (performed by the principal investigator (or designate) once informed consent has been obtained), will be 1:1 RIC:placebo, stratified by use of thrombolysis, and minimised by age (cut 70 years), BP (cut 170 mmHg), sex (M/F), time since stroke (cut 12 hours), stroke severity (NIHSS cut 10).

This approach improves baseline matching and statistical power and ensures concealment of allocation. Attempts are made to keep the patient blinded by using a placebo procedure. Though it will not be possible to blind the research nurse/medic performing RIC (or placebo) during the baseline assessments, subsequent outcome measures will be blinded to treatment allocation. The data monitoring committee (who are un-blinded) will not have any contact with study participants.

Choosing an adequate sham is challenging. If we use inflation pressures that are too high, it may be possible to induce a treatment effect with venous compression. We accept that at the time of cuff inflation, a participant may be able to distinguish between treatment and sham, which was a concern during our pilot trials. Therefore, we tested the adequacy of treatment blinding in both RECAST-1 and RECAST-2 through asking the participants at day 90 (the timing of the primary outcome) which intervention they thought they received. In RECAST-1 (single dose within 24 hours of stroke) 68% participants were wrong (52% didn't know, 16% incorrect); in RECAST-2 (n=60, repeated dosing started within 6 hours of stroke) 93% did not know and 4% were incorrect. This has provided confidence in the sham procedure. Nonetheless, a higher value of 50mmHg has been selected to provide adequate feeling of pressure in the cuff and a figure lower than diastolic arterial pressure. The choice of +20 mmHg above the systolic blood pressure in the treatment arm is selected to enhance patient comfort and tolerance compared to inflating to >200mmHg, which can be uncomfortable.

Multiple efforts will be taken to minimise bias: concealment of allocation, use of sham device identical in appearance to treatment device, blinded central telephone follow-up (eliminating bias from local measurement), blinded adjudication of adverse events and CT scans, analysis by intention-to-treat with adjustment for key prognostic variables. Minimisation on key prognostic variables will help improve precision.[59]

Maintenance of randomisation codes and procedures for breaking code

In general, there should be no need to unblind the allocated treatment since the treatment is completed over 2 weeks and investigators administering RIC or sham are unblinded. Unblinding the participant or the treating doctor (if they are unaware of allocation) should be done only if the doctor believes that clinical management depends importantly upon knowledge of whether the patient received RIC or placebo. Should this be the case, the chief investigator can be contacted to reveal treatment allocation. The date and reasons for unblinding will be recorded in conjunction with routine SAE reporting as appropriate. Upon trial completion and after database lock, treatment allocation will be revealed for statistical analysis.

6.3 TRIAL MANAGEMENT

Trial Management Group will manage the trial on a daily basis and will meet 2 times per month. The group will consist of the CI, trial manager, trial medic, outcome assessor, trial statistician and programmer. The group will monitor trial accrual, centre management (with local CRN research nurses/practitioners) and ensure recruitment strategy remains on target. Centres will be regularly contacted in the event of participant attrition.

Trial Steering Committee will lead the trial strategically, reviewing recruitment rate, data integrity and trial event rates. Any new data emergent from other trials will be discussed for potential impact on RECAST-3. The committee will consist of an independent chair, independent members; the CI and grant holders (observers); PPI representatives; and a sponsor representative. The TSC will meet 6 monthly. As per NIHR guidance, independent members will make up a minimum of 75% of

the voting TSC membership. The minimum quoracy for any TSC meeting to conduct business is 67% (two thirds) of the appointed membership.

Safety and data monitoring committee (DMC)
An independent chair will run the DMC with 2 other independent members. Unblinded data provided by Nottingham CTU statisticians; meetings planned biannually. The Chief Investigator and the DMC can request more meetings if deemed necessary for safety.

6.4 DURATION OF THE TRIAL AND PARTICIPANT INVOLVEMENT

45 months with 33 months recruitment (0.66/centre/month) in 60 centres.

Timeline: 45 months (M)
M0-6: trial set up, centre initiation & training (site initiation will be performed over the telephone as performed in TICH-2 (>100 sites). M3-36: recruitment. M37-39: Final day 90 Follow-ups (primary outcome). M40-45: Data clean & lock, analysis & dissemination

Participant Duration: 90±7 days

Vanguard Phase
The trial will run in two phases, phase 1 over the first 9 months of recruitment. Assuming the success criteria have been met, this will run seamlessly (i.e. without halting recruitment) into the main phase (phase 2) of the trial.

Stop-go decision
The trial will proceed to the main phase at 9 months if 100% of the vanguard phase participants have been recruited (n>160). If 60-99% of target is reached at 9 months, we will review strategies to improve recruitment/follow up and proceed with further monitoring as agreed with the TSC. If <60% of target, and all strategies to improve recruitment/retention have been implemented but not resulted in improvement, the TSC will terminate the study.

End of the Trial
The end of the study will be the last visit of the last participant.

6.5 SELECTION AND WITHDRAWAL OF PARTICIPANTS

Recruitment

A member of the patient's usual care team (which may include investigators) will approach the patient or their consultee/legal representative (where a patient lacks capacity to consent) on admission to the Admissions Unit. The investigator or their nominee (which may include the nurse practitioner), e.g. from the research team or a member of the participant's usual care team, will inform the participant or their nominated representative (other individual or other body with appropriate jurisdiction), of all aspects pertaining to participation in the study. Eligibility can be confirmed and consent obtained by members of the research team (which may include research nurses, research practitioners, research associates and research coordinators) who have local approval to do so and are authorised onto the delegation log with the consent taking role. A medically qualified doctor will be available to answer participant queries on their medical care if needed.

If needed, the usual hospital interpreter and translator services will be available to assist with discussion of the trial, the participant information sheets, and consent forms, but the consent forms and information sheets will not be available printed in other languages.

It will be explained to the potential participant or their consultee/ legal representative that entry into the trial is entirely voluntary and that treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time but attempts will be made to avoid this occurrence. In the event of their withdrawal it will be explained that their data collected so far cannot be erased and we will seek consent to use the data in the final analyses where appropriate.

Eligibility criteria

6.5.1.1 Inclusion criteria:

1) Acute ischaemic stroke (≤24 hours post onset)
2) Spontaneous intracerebral haemorrhage ruled out on baseline clinical neuroimaging; Haemorrhagic transformation of infarction (HTI) HI1, HI2, PH1[60] is permitted
3) NIHSS score 5 - 25 at randomisation
4) Age ≥18 years
5) Mechanical thrombectomy sub-study only (selected sites) – received mechanical thrombectomy ≤24 hours post onset

6.5.1.2 Exclusion criteria:

1) Pre-morbid dependency (modified Rankin Scale, mRS>3); lower level of mRS considered but the primary outcome is assessing a shift in mRS, not a dichotomy.
2) Systolic blood pressure less than 80 mmHg
3) Spontaneous intracranial haemorrhage is the presenting stroke aetiology
4) Haemorrhagic transformation of infarction PH2 (haematoma occupying 30% or more of the infarcted tissue, with obvious mass effect) if known before randomisation. Not excluded or withdrawn if occurs after randomisation
5) Pre-existing diagnosis of dementia
6) Coma (GCS <8)
7) Malignancy, and significant co-morbidity (life expectancy <6 months): factors that will lead to a poor outcome, no matter the intervention
8) Capillary blood glucose <3.0mmol/L; hypoglycaemia sufficient to account for neurological symptoms.
9) Seizure on presentation unless brain imaging identifies evidence of significant brain ischaemia (early ischaemic change or hyperdense vessel on CT scan, or angiography confirmed arterial occlusion); Todd's paralysis can mimic stroke.
10) Significant tissue injury of the upper limbs, which in the opinion of the investigator, will be exacerbated by remote ischaemic conditioning,
11) Taking part in another interventional trial, unless co-enrolment has been approved by both

Chief Investigators and Sponsors. Co-enrolment in observational studies is generally accepted. A separate file is generated for the trials where co-enrolment has been agreed.

12) Known pregnancy – whilst RIC is not expected to be harmful, there are no data currently to support this.

Note: "A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. Acceptable contraceptive methods include: established use of oral, injected or implanted hormonal methods; placement of an intrauterine device (IUD) or intrauterine system (IUS); condom or occlusive cap (diaphragm or cervical/vault caps) with spermicide; true abstinence (when this is in line with the preferred and usual lifestyle of the participant); or vasectomised partner. Where pregnancy cannot be excluded on the basis of the above or is difficult to ascertain (participant lacks capacity and consultee does not know) then a pregnancy test shall be carried out."

13) Expected repatriation of the participant to another hospital not participating in RECAST-3 where RIC or sham cannot continue.

14) Mechanical thrombectomy sub-study only (selected sites) – known contra-indication to administration of iv contrast (required for a CT Perfusion scan). This may include previous allergic reaction to contrast or considered high risk for contrast induced nephropathy (at the discretion of the investigator).[61] This is not an exclusion for the main trial.

Expected duration of participant participation

Study participants will be participating in the study for 90±7 days.

Removal of participants from therapy or assessments

Participants may be withdrawn from the trial either at their own request or at the discretion of the Investigator (e.g. due to safety reasons, failure of participant to adhere to protocol requirements, disease progression, withdrawal of consent). The participants will be made aware that this will not affect their future care. Participants will be made aware (via the information sheet and consent form) that should they withdraw, the data collected to date cannot be erased and may still be used in the final analysis. Efforts will be made to retain subjects in the trial and continue to collect their data (as per the intention-to-treat principle), unless the participant wishes to discontinue their participation in the study altogether. All efforts will be made to record the reason for discontinuation of treatment or trial with detail (e.g. removal of subjects due to an adverse event).

Informed consent

Investigators may obtain oral consent/advice before full written consent/advice in circumstances where written consent/advice cannot be obtained in a timely fashion (as approved and practised in RECAST-2 and other hyperacute stroke trials sponsored by the University of Nottingham (e.g. TICH-2 [62] assessing tranexamic acid administration within 8 hours of stroke onset); rationale being that the sooner the intervention is given, the greater the potential benefit thought to be gained. In RECAST-2, use of initial oral consent compared to written consent resulted in significantly faster time to randomisation by a mean of 84 minutes ($p<0.001$). The following procedure will be used for giving information and obtaining informed consent for RECAST-3:

6.5.5.1 Patient has capacity to provide consent and time allows:

All participants who are able to will provide written informed consent. The Informed Consent Form will be signed and dated by the participant before they enter the trial. The Investigator (or nominee) will explain the details of the trial and provide the Participant Information Sheet. The Investigator will answer any questions that the participant has concerning study participation. Potential participants will be given as long as they need to consider whether to consent, however we recommend that a decision is made as soon as possible due to potential time-dependent effects. It will be explained to the potential participant that as this is an emergency treatment, with a small therapeutic time window. If the participant is unable to write (e.g. in the presence of dominant hand weakness, ataxia or dyspraxia), witnessed verbal consent may be recorded on the consent form.

6.5.5.2 Patient has capacity but time prohibits full written consent:

If the time window does not allow investigators to seek written consent and the attending clinician considers it appropriate, the potential participant will be asked if they are willing to be recruited. Specifically, the responsible investigator will explain to the patient that they will receive the usual care for potential stroke but that in addition to this, the patient can be enrolled in a research study that aims to improve the treatment of patients with this condition. It will be explained that the study is being done to see whether using ischaemic conditioning (5 minute cycles of inflation/deflation of bilateral blood pressure cuffs) will help patients with stroke by protecting the brain from further damage. Further information will be provided on request. If requested, the information sheet will be provided. If they say yes, the potential participant will be randomised **using this initial oral consent** (which could be obtained via video link, such as in some centres using tele-medicine). Full, written informed consent will be sought within the next 24 hours or as soon as is practicable. Written informed consent will be sought for access to medical notes and for participation in the trial follow up. The participant information sheet will be provided to the participant at this time if not already provided. This was the approach used successfully in RECAST-2.

6.5.5.3 Patient lacks capacity to give consent

The participant's attending clinical care team will determine lack of capacity. If the potential participant lacks capacity to give meaningful consent (e.g. in cases of dysphasia, confusion, or reduced conscious level) the following procedure will be employed:

6.5.5.3.1 Consultee/ legal representative (Scotland) present and time allows: If a consultee (relatives or other representative such as partner or close friend, able to represent the patient's presumed views and wishes) is present, bearing in mind the clinical situation and their level of distress, they will be provided with information about the trial. Specifically, the responsible investigator will explain to the consultee / legal representative that the patient will receive the usual care for potential stroke but that in addition to this, the patient can be enrolled in a research study that aims to improve the treatment of patients with this condition. It will be explained that the study is being done to see whether using ischaemic conditioning (5 minute cycles of inflation/deflation of bilateral blood pressure cuffs) will help patients with stroke by protecting the brain from further damage. The consultee will be informed that the patient will have the blood pressure cuffs applied to their arms for 40 minutes whilst the rest of their usual treatment continues, and RIC/sham doses repeated twice daily for 14 days. An information sheet and advice form will be provided. If they say yes, the potential participant will be randomised. Full informed written consent will be obtained from the patient if capacity is regained.

6.5.5.3.2 Consultee / legal representative (Scotland) present but time prohibits full written advice:

If a consultee (relatives or other representative such as partner or close friend, able to represent the patient's presumed views and wishes) is present, but the time window does not allow for full written advice, bearing in mind the clinical situation and their level of distress, they will be provided with brief information about the trial. Specifically, the responsible investigator will explain to the consultee that the patient will receive the usual care for potential stroke but that in addition to this, the patient can be enrolled in a research study that aims to improve the treatment of patients with this condition. It will be explained that the study is being done to see whether using ischaemic conditioning (5 minute cycles of inflation/deflation of bilateral blood pressure cuffs) will help patients with stroke by protecting the brain from further damage. The consultee will be informed that the patient will have the blood pressure cuffs applied to their arms for 40 minutes whilst the rest of their usual treatment continues, and RIC/sham doses repeated twice daily for 14 days. If they say yes, the potential participant will be randomised **using this initial oral consent.** This oral consent could take place over the telephone or video link with the potential participants consultee/legal representative, facilitated by the telephone consent form. Full Consultee Advice will be obtained as soon as practicable. Full informed written consent will be obtained from the patient if capacity is regained.

6.5.5.3.3 Relatives not present: Please note – independent physician consent does not apply to sites in Scotland. In the absence of anyone being found in time (if no relatives are available), we intend to recruit a doctor, unconnected with the trial, provide them with written information

relating to the trial (Consultee information sheet (England)) and obtain their written consent for the patient's inclusion in the trial. The independent doctor will complete the Consultee Declaration Form (England). If a doctor unconnected with the trial is not available, patients will not be entered into the trial. If enrolled, full informed written consent will be obtained from the patient or their consultee/legal representative afterwards as soon as practicable. We feel this is justifiable given that RIC is an urgent treatment, can be given prior or in parallel to urgent reperfusion therapies (thrombectomy or thrombolysis), and there have been no documented significant adverse effects of RIC in multiple other clinical trials of other populations (i.e. patients with heart attacks receiving RIC in the ambulance and the DANISH RESIST trial which administered RIC in the ambulance to presumed stroke patients). **Participants undergoing independent physician consent will NOT be enrolled into either the MT sub-study or co-enrolled into any other trial.**

If oral consent for recruitment has been given, participants (or their consultee/legal representative) will be approached as soon as possible after recruitment to give written consent or advice (where a consultee is involved). If oral/verbal consent is gained remotely, then follow-up written consent (obtained as soon as is practicable) may be obtained through the post or via email, whichever is most convenient to the investigators, participants and their consultees/legal representatives. During the process of recruitment and randomisation, the type of consent taken will be documented and monitored to ensure all those with initial oral consent are followed up with written consent.

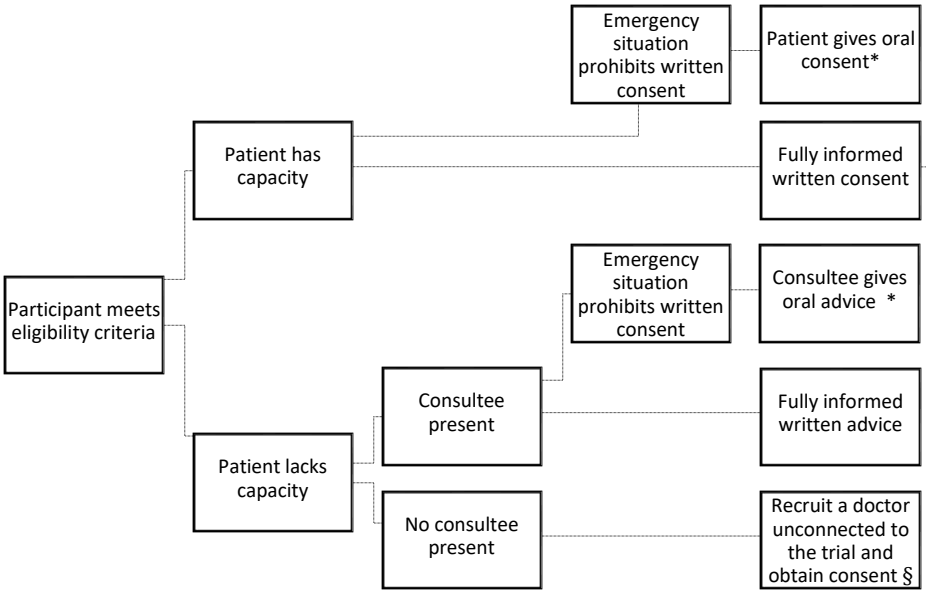
Where the patient is being assessed and treated via telemedicine (as is often standard care in many stroke services out of hours) by a member of the medical team who is appropriately trained and listed on the delegation log, the process is as above, with the exception that the paper consent form will be countersigned by a witness, and signed by the investigator upon their return to the hospital site. If the patient does not wish to decide via telemedicine they will not be enrolled.

Participants who originally lacked capacity (and were entered into the study following agreement from a consultee) but then regain capacity will need to give informed written consent to continue in the study. The participants' decision to withdraw would overrule the decision of the consultee.

One copy of the consent form will be kept by the participant, one will be kept by the Investigator, and a third will be retained in the patient's hospital records.

Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the trial, continuing consent will be obtained using an amended Consent form which will be signed by the participant.

Consent process:



Patients or the consultee (legal representative in Scotland) will be approached to give oral advice in circumstances where the therapeutic time window does not allow investigators to seek full informed written consent, and only if the attending clinicians consider it appropriate. Patients or consultees will not be approached if there is insufficient time to give a brief oral summary of the trial, or they do not speak fluent English and no translator is available. If the patient or relative (consultee) does not give oral consent/advice they will not be recruited.

* If oral consent for recruitment is given, participants (or consultee) will be approached as soon as possible after recruitment to give written consent/advice (where a consultee is present).

§Where Patients who lack capacity to consent (e.g. severe dysphasia) and have no relative present their enrolment will be discussed with a doctor unconnected to the trial. Information relating to the trial will be provided using the relative information sheet and their written consent for the patient's inclusion in the trial obtained. If a doctor unconnected with the trial is not available, patients will not be entered. **Independent physician consent does not apply to sites in Scotland.**

7 TRIAL TREATMENT AND REGIMEN

We have selected a dose of RIC based on (i) cycle number and duration of limb ischaemia seen to reduce infarct volume in meta-analysis of pre-clinical stroke models;[18] (ii) combining both per and post conditioning appears more effective than per conditioning alone;[20] and (iii) information from recent RICAMIS and RESIST trials (see background).

7.1 INTERVENTION DELIVERY

The investigator (which may include a trained research nurse/practitioner) will inspect the limbs and skin condition and make note of any pre-existing skin changes or damage. The cuffs will be placed on both upper arms to deliver RIC or sham. The participant's blood pressure will be checked and recorded. The investigator will set the inflation pressure either to the RIC or sham protocol as described below, depending on the randomisation result. Treatment will occur immediately after randomisation as practised in RECAST-2. This process should not delay door-to-needle times or the need for mechanical thrombectomy.

A trained research nurse/practitioner at each centre will be allowed to take consent and deliver the intervention/sham:

- **Active:** RIC group: 4 cycles of intermittent limb ischaemia - alternating 5 minutes inflation (+20 mmHg above systolic BP) followed by 5 minutes deflation of bilateral upper arm blood pressure cuffs.
- **Control:** Sham RIC. Bilateral upper arm blood pressure cuffs are inflated to 50 mmHg for 4 cycles (5 minutes inflation/5 minutes deflation).

Duration of treatment:

- First dose (4 cycles of RIC or sham) within 24 hours of onset.
- Twice daily doses (4 cycles RIC/sham) until received 14 days (28 'doses') of treatment;

Notes:

- Since the AneticAid device pressures increase in 5 mmHg increments, RIC treatment cuffs should be inflated to at least 20 mmHg above systolic BP, to the nearest 5 mmHg. For example, if systolic BP is 140 mmHg, the target is 160 mmHg; if systolic BP is 141-145mmHg, the target is 165mmHg.
- Some centres will be unable to administer RIC over a weekend due to absence of trained staff. In these cases we accept RIC/sham may be omitted over the weekend so long as they have already received a minimum of 48 hours of RIC/sham (i.e. 4 x 4 cycles).
- A minimum of 4 hours is required between twice daily dosing. If randomisation occurs late on day 1, not allowing a second dose on day 1, then dose 2 occurs on day 2.
- If a participant omits dosing due to a weekend, the total number of RIC doses should remain 28. For example, if 4 days (8 doses) are omitted over 2 weekends, then total treatment time may be over 18 days.
- If a participant is due to be discharged or transferred to another facility that cannot deliver the trial treatment (i.e. not a RECAST-3 participating site) prior to the full treatment course being completed, RIC/sham is discontinued.
- If a dose is omitted due to treatment intolerance, there is no need to extend the treatment period but further doses at the usual timepoints will be offered.

Device

The device cuffs are placed on the upper arms and require the investigator to set the inflation pressure and attend to the device every 5 minutes to elicit the periods of inflation and deflation. The same device will be used to deliver both RIC or sham protocols.

In the event of a device failure, the intervention will be discontinued and an alternative device used (if available). The faulty device will be returned to the trial coordinating centre and investigated through the adverse event reporting system. The manufacturer will investigate the faulty device. If the device is unavailable (e.g. due to use for another participant), the RIC or sham procedure can be carried out using manual sphygmomanometers, as performed in RECAST-1 and RECAST-2. Any manual sphygmomanometer that is used in routine clinical practice can be used. At the

bedside, the attending investigator can manually inflate and deflate the BP cuffs on the upper arms to the desired pressures as per the cycles described above.

Other treatment

All patients will receive standard stroke unit guideline care as per local investigator stroke unit policy; treatment deemed appropriate may include thrombolysis, mechanical thrombectomy, hemi-craniectomy, admission to a stroke unit, secondary prevention (anti-platelets, statins, anti-hypertensives, carotid endarterectomy), prevention of complications (e.g. intermittent compression stockings, antibiotics) and therapy (physical, occupational, speech/swallow).

On arrival to hospital, the patient will be screened for eligibility for the trial by a member of their usual care team (who may be a member of the research team). Should they fit the criteria, they (or their relative/carer) will be enrolled into the trial according to the consent process above. Should they agree to the trial and give consent/advice, the following will occur:

7.2 FOLLOW-UP

Day 1
Following consent and randomisation, baseline routine clinical assessments will be conducted, including pre-intervention BP. If taking part in the mechanical thrombectomy sub-study, the patient will have a CT perfusion scan prior to the trial intervention. The patient will then receive RIC or placebo using the device. A blood pressure measurement after RIC/placebo is taken. A second dose of 4 cycles is applied >4 hours after the end of the first dose if time allows in the day.

Day 2
Two further doses of RIC or sham are applied, once in the morning and once in the afternoon. A further neurological (NIHSS) and safety assessment will be conducted.

End of RIC/Sham treatment period (or on discharge if earlier)
At the end of 28 doses (of 4x RIC/sham cycles) a clinical assessment is performed including (NIHSS and safety (e.g. new outcome events). This will be performed in hospital. See notes in section 7.1 regarding omitted doses.

Day 2-14 (selected sites only)
Only participants in the mechanical thrombectomy sub-study will have a MRI at Day 2-14. (Local sites will need to arrange return for the scan if the participant has already been discharged)

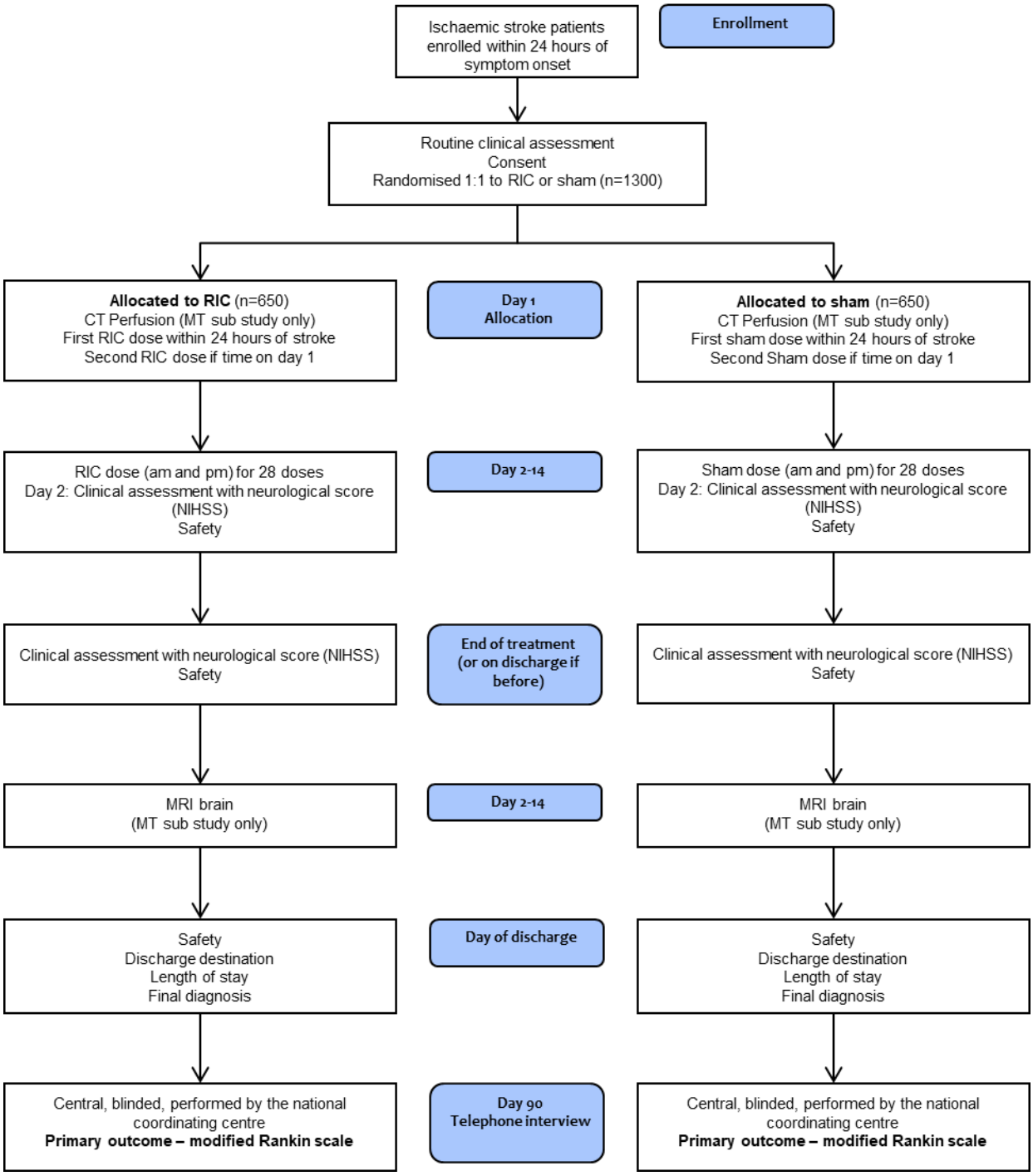
Discharge or death
Information provided on final diagnosis, length of stay, discharge destination, clinical scans for stroke phenotyping, and secondary outcome data collection

Day 90 (±7)
Researchers will first contact the participants general practitioner (GP) at Day 90 to check the patient's health status. Permission to contact the GP at day 90 will be sought at the time of consent. Telephone contact will then be made with the patient or consultee asking questions regarding level of function, activities of daily living, mood, cognition, quality of life, frailty, outcome events and re-admissions (and reason). If the patient cannot be contacted, then a postal version of the questions will be sent to the patient or consultee.

Day	**Pre-enrolment**	**1**	**2**	**2-14†**	**2-14**	**14**	**90**
All patients:							
Consent		X					
CT head scan	X *						
CT Perfusion (selected centres only)		X					
RIC /sham		X X	X X	X X			
Clinical efficacy:							
Impairment: (NIHSS)		X *	X			X	
Day 90 outcomes via telephone							X
Safety		X	X			X	X
MRI brain					X *		

RIC, remote ischaemic conditioning; NIHSS, National Institutes of Health Stoke Scale; * Performed as part of routine clinical care. † RIC/sham over 14 days (28 doses)

RECAST-3 Trial Flow



Data collection at baseline
Local investigators are to collect and enter data over the trial's secure internet site prior to randomisation. Data collection is kept to a minimum in order to facilitate rapid enrolment and treatment. Data includes the randomisation variables. After randomisation there is an additional data collection form that includes: ethnicity, pre-morbid dependency and frailty, and medical history. This will be collected within the first 24 hours but does not need to be done prior to randomisation in order to allow rapid treatment.

Data collection at follow-up
Local investigators will collect and enter data and images over the secure internet link after randomisation: The day after randomisation (Day 2): neurological impairment (NIHSS) SAEs, intervention safety and compliance; End of treatment RIC or sham (or on discharge if earlier): neurological impairment (NIHSS), SAEs, intervention safety and compliance; on death or discharge: length of stay, disposition; and uploading of neuroimages. The National Coordinating Centre are to collect information (blinded to treatment allocation) on primary and secondary outcomes at day 90 (end of follow-up) by telephone (following a check with the general practitioner to verify vital status and current address). Participants will be 'flagged' with NHS Digital (or as known by any future name) to confirm death.

Neuro-imaging data collection
As part of standard care, all participants will have had a baseline CT scan on admission to hospital (prior to enrolment) to rule out intracranial haemorrhage or other stroke mimic. Administration of intravenous contrast, CT angiography (CTA), MRI or MR angiography (MRA) will be performed if part of the centre's local practice. Investigators will submit basic information on imaging (presence of new infarct, mass effect, intracranial haemorrhage, atrophy, white matter disease) as read locally for CT scans performed at baseline, 24 hours after randomisation (if clinically indicated, e.g. post-thrombolysis) and for all additional clinical brain scans done during the 90 day follow up period. Baseline CT (including any contrast-enhanced scans and MRI), follow-up CT scans, and day 2-14 MRI (from MT sub-study) will be collected (encrypted DICOM data via internet or via posted CD) for all patients to allow adjudication by a neuroradiologist) blinded to treatment so that accurate and consistent imaging phenotyping is available, particularly in respect of swelling of the original infarct and cerebral oedema, infarct volume, new haematoma (parenchymal, petechial, intraventricular, remote).

Compliance
The investigator administering RIC will make assessments of compliance and cuff tolerance. Data will be collected on the length of time that the cuffs are tolerated and the number of cycles completed. The investigator will log the date and dose administered on each use.
Any protocol violations will be recorded, for example if a patient is randomised into the study and does not receive RIC or sham.

7.3 RADIATION EXPOSURE

Details of diagnostic or therapeutic ionising radiation
Participation will receive a routine CT head scan (and possibly CT angiography) at the time of presentation with stroke, before trial enrolment and another CT head scan on day 2 if clinically indicated (e.g. post thrombolysis). The CT head scan at the time of stroke is part of routine clinical care whether or not the patient goes on to participate in the trial. For patients who are randomised into the trial, the results will be used as baseline data. For those taking part in the MT sub study, an additional CT (perfusion) brain scan is obtained in conjunction with the baseline CT brain scan or as soon as possible after this, prior to the trial intervention.

Details of radioactive materials and dose
A CT of the brain will give an average of 1.9mSv effective dose. The published effective dose for a CTP brain varies from 3-8mSv.

Considering 1 CT brain and 1 CTP brain as additional, as the worst-case-scenario, the effective dose through trial participation might be around 10mSv. The actual doses received will vary with equipment characteristics and patient size, so a margin of uncertainty is applied to these values. The estimated total research protocol dose therefore is approximately 15mSv, all of which would be additional through participation.

Risk Assessment (induction of fatal cancer)
The stochastic risk resulting from radiation exposure is that of inducing a fatal cancer at a later date. The median latency period for leukaemia induction is 8 years, and two to three times that for solid tumours. The participants in this trial are assumed to have full life expectancy.
Using the estimated risk coefficient for radiation exposure in healthy adults of 5% per Sievert, the total exposure through participation in this trial is estimated to increase the risk of inducing fatal cancer by around 1 in 1300 (or 0.075%). This is categorised as moderate in International Commission on Radiological Protection (ICRP) report 62. All of this may be additional through trial participation, the equivalent of around 6 years of additional background radiation.

Clinical Assessment
The scan itself takes about half a minute and does not involve any injections. The scan uses x-rays, which in large amounts can be harmful, but for this extra CT head scan the additional risk to you from the scan has been judged to be extremely small. If taking part in the MT substudy, the CT perfusion scan takes a similar amount of time but also includes an injection of contrast before the scan. The following will be explained to the participant: The objective of the exposure is to assess the extent of the stroke on the brain to see if it has got worse (larger) or better (smaller) following treatment. An alternative would be MRI brain scan but this takes longer and many patients are unsuitable or unable to tolerate it due to claustrophobia. The objective of a CTP would be to obtain a more precise estimate of the size of the stroke and help determine if the trial treatment is having an effect.

The procedure for CT and any doses in lay terms are explained in the participant information sheet.

8 STATISTICS

Sample size
1300 adults (650 RIC, 650 Sham) with AIS presenting to 60 Stroke Services in the UK

Main trial
We expect a majority of patients in the main trial to receive reperfusion therapy alongside RIC/sham (RECAST-2, 55% received thrombolysis). Pre-clinical models of RIC reduced infarct volume, by 35%,[18] greater than the 25% reduction in pre-clinical thrombolysis studies [63] and, experimentally, RIC in combination with thrombolysis has additive effects.[21] In merged data from our pilot studies RECAST-1 and RECAST-2 (n=86, post-hoc analyses), common odds ratio for a poor outcome, adjusted for baseline stroke severity, is 0.83 (95%CI 0.39-1.75), (lower ORs indicating a better outcome). If participants with diabetes are excluded (diabetes is recognised to diminish the effects of RIC), the OR of a poor outcome is 0.76 (95%CI 0.33-1.77).

Calculation
The null hypothesis (H_0) is that RIC does not alter death or dependency in participants with acute ischaemic stroke. The alternative hypothesis (H_A) is that death or dependency improves in those participants randomised to RIC compared to sham.

The sample size for RECAST-3, based on a 7-level ordinal shift analysis, has assumed an end-of-trial mRS distribution from the UK based IST-3 (n=3035) where 50% received alteplase [64] and were randomised within 6 hours of ischemic stroke (mRS 0-6: % 8.5 / 14 / 13.5 / 14.5 / 8.5 / 14.5 / 27). Other assumptions: alpha=0.05, power=90%, losses to follow up=5% (<1% in ENOS[8] & TICH-2[62]) & covariate adjustment reducing sample size by 20%;[65] a sample size of 1300 will be needed to detect an OR 0.75 (0.78 with 80% power) by shift analysis of mRS (Table below), which lies in the range seen in related acute stroke trials**.** The ordinal odds ratio refers to the odds of a lower score (i.e. improvement) on the mRS by one or more points in the RIC arm compared to the sham arm.

Odds ratio	Odds ratio	RRR (%)	Each group	Total trial	With covariate adjustment	& losses to follow up
Binary	**Ordinal**		**N**	**N**	**N**	**N**
0.55	0.60	22.7	250	500	400	420
0.60	0.65	19.3	351	702	562	591
0.66	0.70	15.6	512	1024	820	861
0.67	0.71	15.0	556	1112	890	935
0.68	0.72	14.4	604	1208	967	1016
0.69	0.73	13.9	658	1316	1053	1106
0.70	0.74	13.3	719	1438	1151	1209
0.71	**0.75**	12.8	**787**	**1574**	**1260**	**1323**
0.72	0.76	12.2	865	1730	1384	1454
0.73	0.77	11.7	954	1908	1527	1604
0.75	0.78	10.7	1055	2110	1688	1773
0.76	0.79	10.2	1172	2344	1876	1970
0.77	0.80	9.7	1308	2616	2093	2198
0.82	0.85	7.3	2466	4932	3946	4144
0.88	0.90	4.7	5866	11732	9386	9856

Note 1: The mRS ranges from 0 (no symptoms) to 6 (death). The mRS will be analysed as an ordinal scale (using all 7 levels) rather than a binary scale (e.g. 0-2 vs. 3-6) as this will improve statistical power.[65] A binary outcome (0-2 vs. 3-6) would require 1,773 participants for the same assumptions.

Note 2: The ordinal odds ratio refers to the odds of a lower score (i.e. improvement) on the mRS by one or more points in the RIC arm compared to the sham arm.

Note 3: For comparison, the OR for death and dependency in recent stroke trials have reported the following unadjusted odds ratios for between-group comparisons based on ordinal analysis (in OR order): Hemicraniectomy OR=0.16 (n=93),[66] NINDS-alteplase 0-3hours OR 0.60 (n=624);[67] ECASS-3/alteplase 3-4.5hours OR=0.79 (n=821),[68] IST-3/alteplase 0-6hours OR=0.85 (n=3035), SCAST/candesartan OR=0.85 (n=2029),[69] INTERACT-2/intensive BP-lowering ICH OR=0.87 (n=2794), IST-1/aspirin OR=0.95 (n=19435),[70] RIGHT-2 OR 1.25 (n=1149).[71]

Note 4: The Danish RESIST trial (NCT03481777) had a sample size target n=1500 (1000 (67%) acute ischaemic stroke and ICH, 400 (27%) stroke mimic, 60 DWI negative TIA (4%), and 30 (2%) lost to follow up), based on a 6-7% absolute improvement in the modified Rankin Scale.[72] Data from the RESCUE BRAIN investigators derived from 188 participants, in whom RIC was applied to the leg within 6 hours of stroke, showed the proportion of participants in the RIC group with a day 90 mRS 0-1 was 8.3% better than control (48.6% v 40.3%, p=0.26), equivalent to a RRR 20.6%.[73] RECAST-2 (n=60) RRR was 14% (mRS 3-6 40% v 46.6%, p=0.46). Overall, a 20% RRR feels optimistic, and our larger sample size ensures we won't be missing smaller treatment effects up to RRR 12.8%.

Note 5: The RICAMIS trial suggested a positive treatment effect with an acOR 1.37 (95% CI, 1.16-1.63, *P* <0.001) in favour of RIC, equivalent to odds of a poor outcome of 0.73, requiring a sample size of c1100.

In summary, a trial of 1,300 participants will have 90% power to detect an ordinal shift of mRS outcome with odds ratio 0.75.

Assessment of performance

Treatment groups will be compared on an intention-to-treat basis in the primary analysis but also, secondarily, a per protocol set excluding participants with a final diagnosis that is non-stroke (a stroke mimic) and those with major protocol violations. Safety analyses will be performed on the safety population.

Primary analysis: 'Ordinal Shift' in day 90 mRS between treatment and control groups using ordinal logistic regression (OLR, following a check for proportionality of odds) with adjustment for minimisation variables & other pre-specified prognostic baseline factors. These analyses are now routinely performed in large acute stroke trials,[50] as we have done previously.[8,62]

Safety Analyses. Safety analyses will be performed 6 monthly by the Data Monitoring Committee.

Secondary outcomes will be analysed using: Kaplan-Meier curve/Cox regression (time to first cerebrovascular event/death/MACCE/AKI); logistic regression (binary events/individual components of composite, SAEs); multiple regression (continuous variables); repeated measures ANOVA (BP, heart rate, derivatives); these analyses will be covariate adjusted. A separate statistical analysis plan will be published prior to completion of recruitment.

Planned subgroup analysis

Pre-specified subgroup analyses in all minimisation variables, including: age (≤70/>70); sex; time to randomisation (0-6hours, 6-12hours, 12-24hours); severity (NIHSS <10, 10-20, >20), new diabetes (yes/no); systolic BP (≤170/>170mmHg); pre-morbid frailty (CFS none/mild/moderate) vascular location (anterior v posterior); thrombectomy (yes/no); alteplase (yes/no); aetiology (embolic/large vessel vs small vessel). Analysis of the primary outcome in these pre-specified sub-groups does not comprise the primary analysis and has not informed the sample size calculation. The interpretation of any subgroup effects will be based on interaction tests.

Criteria for terminating trial

The DMC will monitor outcomes and SAEs and can recommend stopping or altering the trial, through asymmetric stopping rules, on the basis of safety and efficacy. A DMC Charter will be prepared with full details of stopping guidelines. In brief, the trial would be stopped if shift analysis of mRS favours the active or control group with P<0.001 (2-sided). The significance level of

P<0.001 amounts to ‘proof beyond reasonable doubt’. Further decisions to terminate the trial could be based on poor accrual rate despite remedies to identify barriers of recruitment. RECAST-1 and 2 demonstrated excellent treatment compliance; nonetheless compliance and safety will be closely monitored through the trial steering committee (TSC) and DMC. Safety analyses will be performed 6 monthly.

Procedures for missing, unused and spurious data

Missing data will be reported, rules/methods for handling missing data will be detailed in the statistical analysis plan. Experience from our previous acute stroke trials indicates that missing data on the primary outcome (mRS) are unusual (ENOS <1%, TARDIS <1%, TICH-2 <1%, RECAST-2 0%). However, multiple imputation will be used as a sensitivity analysis.

Definition of populations analysed

Safety population: All randomised participants.
Intention-to-treat population: All randomised participants, who receive at least one dose of study medication. The intention-to-treat population will be defined in a blinded review prior to database lock.

Per protocol population: All participants in the intention-to-treat population who are deemed to have no major protocol violations that could interfere with the objectives of the study. The per-protocol population will be defined in a blinded review prior to database lock. The per protocol set will also exclude participants with a final diagnosis that is non-stroke (a stroke mimic).

Analyses

All efficacy analyses will be performed on the intention-to-treat population; the robustness of the primary and key secondary analyses will be assessed in the per-protocol population. Safety analyses will be performed on the safety population.

Protocol Violation

A protocol violation is a major deviation from the trial protocol where a participant is enrolled in spite of not fulfilling all the inclusion and exclusion criteria, or where deviations from the protocol could affect the trial delivery or interpretation significantly.

The following baseline characteristics constitute a protocol violation:

1. Failure to obtain appropriate consent prior to randomisation
2. Randomisation > 24 hours from onset of symptoms
3. Systolic blood pressure less than 80mmHg
4. Participant less than 18 years of age
5. Spontaneous intracerebral haemorrhage present on baseline clinical neuroimaging (haemorrhagic transformation of is permitted)
6. NIHSS score <5 or >25 at randomisation
7. Pre-existing dementia
8. Coma (GCS <8)
9. Malignancy
10. Significant injury of the upper limbs
11. Known probable life expectancy of less than 3 months
12. Capillary blood glucose <3.0mmol/L
13. Seizure on presentation when brain imaging does not confirm evidence of significant brain ischaemia (early ischaemic change or hyperdense vessel on CT scan, or angiography confirmed arterial occlusion)
14. Pregnancy

The following practice during the trial constitutes a protocol violation:

1. Subsequent randomisation into another drug or devices trial unless this has prior agreement from both CIs and Sponsors
2. Patient does not receive randomised treatment
3. Failure to complete SAEs where appropriate
4. Failure to complete outcomes where appropriate
5. Follow-up assessments are performed (as opposed to submitted) outside the specified time as shown below:
    a. 2-day follow-up: >2 days past the due date
    b. End of treatment follow-up: >7 days past the due date
    c. Discharge or death in hospital form: >30 days past the due date
    d. 90-day follow up: >30 days past the due date

Protocol Deviation
A Protocol Deviation is a minor deviation from the protocol that affects the conduct of the trial in a minor way. This includes any deviation from the trial protocol that is not listed as a Protocol Violation. Examples of Deviations are given below but this is not exhaustive.

Follow-up assessments are performed (as opposed to submitted) outside the specified time as shown below:
a. 2-day follow-up: >1 day past the due date
b. End of treatment follow-up: >2 days past the due date
c. Discharge or death in hospital form: >7 days past the due date
d. 90-day follow-up: >7 days past the due date

Review of Protocol Violations and Deviations
Protocol Violations will be reviewed annually by both the Data Monitoring Committee (using unblinded data) and the Trial Steering Committee (with blinding to treatment assignment).

The list of protocol violations and deviations will be updated, as necessary, in a working practice document which will be uploaded and available on the trial website.

9 ADVERSE EVENTS

Definitions
An adverse event is any unfavourable and unintended sign, symptom, syndrome or illness that develops or worsens during the period of observation in the study.
An AE does include a / an:

1. Exacerbation of a pre-existing illness.
2. Increase in frequency or intensity of a pre-existing episodic event or condition.
3. Condition detected or diagnosed after medicinal product administration even though it may have been present prior to the start of the study.
4. Continuous persistent disease or symptoms present at baseline that worsen following the start of the study.

An AE does not include a / an:
1. Medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); but the condition that led to the procedure is an AE.
2. Pre-existing disease or conditions present or detected at the start of the study that did not worsen.
3. Situations where an untoward medical occurrence has not occurred (e.g., hospitalisations for cosmetic elective surgery, social and / or convenience admissions).
4. Disease or disorder being studied or sign or symptom associated with the disease or disorder unless more severe than expected for the participant's condition.
5. Overdose of concurrent medication without any signs or symptoms.

Adverse Device Effects
An adverse device effect is defined as any untoward and unintended response to a medical device and includes any event resulting from insufficiencies or inadequacies in the instructions for use or the deployment of the device and any event that is a result of a user error.

Serious Adverse Events
A Serious Adverse Event (SAE) is any adverse event occurring following study mandated procedures, having received the treatment or intervention that results in any of the following outcomes:

1. Death
2. A life-threatening adverse event
3. Inpatient hospitalisation or prolongation of existing hospitalisation
4. A disability / incapacity
5. A congenital anomaly in the offspring of a participant

Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

All adverse events will be assessed for seriousness, expectedness and causality:

A distinction is drawn between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined using the criteria above. Hence, a severe AE need not necessarily be serious.

The following events are considered as safety or secondary end points, not SAEs per se:

- death
- recurrent ischaemic stroke, transient ischaemic attack (TIA)
- intracranial haemorrhage, defined using the Heidelberg bleeding classification[60]
- symptomatic swelling of the original infarct[6]
- neurological deterioration
- systemic embolism
- neurovascular limb compromise secondary to RIC
- myocardial infarction
- AKI

All SAEs during the RIC/sham treatment period (i.e. ≤20 days after randomisation) will be collected. SAEs after the RIC/sham treatment period (i.e. >20 days after randomisation) will not be collected; thereafter, only fatal SAEs and safety outcomes will be recorded and blindly adjudicated until day 90.

Serious Adverse Device Effects
A **Serious Adverse Device Effect (SADE)** is defined as an adverse device effect that resulted in any of the consequences, characteristic of a serious adverse event or that might have led to any of these consequences if suitable action had not been taken or intervention had not been made or if circumstances had been less opportune. Note that this definition captures “near misses” as well as actual incidents.

An **unexpected adverse device effect** is any adverse device effect, the specificity or severity of which is not consistent with the current investigator’s brochure.

Causality

Not related or improbable: a clinical event including laboratory test abnormality with temporal relationship to trial treatment / intervention administration which makes a causal relationship incompatible or for which other treatments, chemicals or disease provide a plausible explanation**.** This will be counted as "unrelated" for notification purposes.

Possible: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment / intervention administration which makes a causal relationship a reasonable possibility, but which could also be explained by other interventions, chemicals or concurrent disease. This will be counted as "related" for notification purposes.

Probable: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment / intervention administration which makes a causal relationship a reasonable possibility, and is unlikely to be due to other interventions, chemicals or concurrent disease. This will be counted as "related" for notification purposes.

Definite: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment / intervention administration which makes a causal relationship a reasonable possibility, and which can definitely not be attributed to other causes. This will be counted as "related" for notification purposes.

With regard to the criteria above, medical and scientific judgment shall be used in deciding whether prompt reporting is appropriate in that situation.

Reporting of adverse events

All adverse events (AEs) will be recorded as they are reported by the participant whether spontaneously volunteered or in response to questioning about well being at trial visits. The questioning about AEs will cover the current visit as well as the period of time between the previous and the current visit. A note of any concomitant medication will also be made so that a full assessment of the AE can be made.

Abnormal laboratory test results that are deemed clinically significant by the investigator and that lead to a change or temporary or permanent discontinuation in the use of the device, or require intervention or diagnostic evaluation to assess the risk to the subject will be recorded as adverse events or adverse device effects in the CRF and instigate further investigation and follow up as appropriate.

All AEs, SAEs, ADEs and SADEs will be documented in the subject's medical records and CRF. All events must be followed until resolution, or for at least 30 days after discontinuation in use of the device, whichever comes first.

Participants will be asked to contact the study site immediately in the event of any SAEs or SADEs. within 20 days of randomisation, after which only outcome events are reported until final follow up at day 90. Non-serious AEs and ADEs do not need to be reported to the trial centre. The Chief Investigator shall be informed immediately of any serious events and shall determine seriousness and relationship in conjunction with any treating medical practitioners.

In the event of a pregnancy occurring in a trial participant monitoring shall occur during the pregnancy and after delivery to ascertain any trial related adverse events in the mother or the offspring.

All reported serious adverse events and serious adverse device effects will be recorded and reported to the REC as part of the annual reports.

SAEs and SADEs will be reported within the statutory timeframes to the REC and Anetic Aid Ltd as stated below. The Chief Investigator will be responsible for all adverse event reporting.

The Chief Investigator will:

- Assess the event for seriousness, expectedness and relatedness to the trial device.
- Take appropriate medical action, which may include halting the trial and inform the Sponsor of such action.
- Report events to Anetic Aid Ltd
- If the event is deemed serious, related and/or unanticipated to the trial device, shall inform the REC using the reporting form found on the NRES web page within 15 days of knowledge of the event.
- Shall, within a further eight days send any follow-up information and reports to the REC.
- Make any amendments as required to the study protocol and inform the REC as required

Participant removal from the study due to adverse events
Any participant who experiences an adverse event may be withdrawn from the study at the discretion of the Investigator.

10 ETHICAL AND REGULATORY ASPECTS

10.1 ETHICS COMMITTEE AND REGULATORY APPROVALS

The trial will not be initiated before the protocol, informed consent forms and participant and GP information sheets have received approval / favourable opinion from the Research Ethics Committee (REC), the respective National Health Service (NHS) or other healthcare provider's Research & Development (R&D) department, and the Health Research Authority (HRA) if required. Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent forms and participant and GP information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC and R&D departments. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the R&D and REC are notified as soon as possible and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of Good Clinical Practice, in accordance with the Medicines for Human Use Regulations, Statutory Instrument 2004, 1031 and its subsequent amendments, the UK Department of Health Policy Framework for Health and Social Care, 2017and the Medical Device Directive.

10.2 INFORMED CONSENT AND PARTICIPANT INFORMATION

The process for obtaining participant informed consent or advice will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The participant or consultee shall provide Informed Consent Form before the person can participate in the study.

The participant will receive a copy of the signed and dated forms and the original will be retained in the Trial Master File. A second copy will be filed in the participant's medical notes and a signed and dated note made in the notes that informed consent was obtained for the trial.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No trial-specific interventions will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the course of the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Informed Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

10.3 RECORDS

Device accountability

Device supplies will be kept under the storage conditions specified by manufacturer.

The investigator and the local site staff shall maintain records of the study devices delivery to the site, an inventory at the site, the distribution to each participant, and the return to the storage or alternative disposition of unused study devices (if applicable). These records will include dates and the unique code numbers (patient trial number) assigned to the trial participant. These records will be part of each patient's Case Report Form (CRF). All study devices received by the site shall be accounted for.

Case Report Forms
Each participant will be assigned a trial identity code number, allocated at randomisation if appropriate, for use on CRFs other trial documents and the electronic database. The documents and database will also use their initials (of first and last names separated by a hyphen or a middle name initial when available) and date of birth (dd/mm/yy) to permit accurate linkage of research data and sample analysis.

CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number, and Participant Trial Number (the Trial Recruitment Log), to permit identification of all participants enrolled in the trial, in accordance with regulatory requirements and for follow-up as required
CRFs shall be restricted to those personnel approved by the Chief or local Principal Investigator and recorded on the 'Trial Delegation Log.'

All paper forms shall be filled in using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated.
The Chief or local Principal Investigator shall sign a declaration ensuring accuracy of data recorded in the CRF.

Source documents
Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, current medical records, laboratory results and records. A CRF may also completely serve as its own source data. Only trial staff as listed on the Delegation Log shall have access to trial documentation other than the regulatory requirements listed below.

Direct access to source data / documents
The CRF and all source documents, including progress notes and copies of laboratory and medical test results shall be made available at all times for review by the Chief Investigator, Sponsor's designee and inspection by relevant regulatory authorities.

10.4 DATA PROTECTION

All trial staff and investigators will endeavour to protect the rights of the trial's participants to privacy and informed consent, and will adhere to the Data Protection Act, 2018. The CRF will only collect the minimum required information for the purposes of the trial. CRFs will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the trial staff and investigators and relevant regulatory authorities (see above). Computer held data including the trial database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one way encryption method).
Information about the trial in the participant's medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information.

Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

11 QUALITY ASSURANCE & AUDIT

11.1 INSURANCE AND INDEMNITY

Insurance and indemnity for trial participants and trial staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but trial participants may have recourse through the NHS complaints procedures.

The University of Nottingham as research Sponsor indemnifies its staff with both public liability insurance and clinical trials insurance in respect of claims made by research participants.

11.2 TRIAL CONDUCT

Trial conduct may be subject to systems audit of the Trial Master File for inclusion of essential documents; permissions to conduct the trial; Trial Delegation Log; CVs of trial staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, correct randomisation, timeliness of visits); adverse event recording and reporting; accountability of trial materials and equipment calibration logs.

11.3 TRIAL DATA

Monitoring of trial data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Trial Coordinator, or where required, a nominated designee of the Sponsor, shall carry out monitoring of trial data as an ongoing activity.

Entries on CRFs will be verified by inspection against the source data. A sample of CRFs (10% or as per the study risk assessment) will be checked on a regular basis for verification of all entries made. In addition, the subsequent capture of the data on the trial database will be checked. Where corrections are required, these will carry a full audit trail and justification.

Trial data and evidence of monitoring and systems audits will be made available for inspection by REC as required.

11.4 RECORD RETENTION AND ARCHIVING

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Nottingham Research Code of Conduct and Research Ethics, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The Trial Master File and trial documents held by the Chief Investigator on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all trial databases and associated meta-data encryption codes.

11.5 DISCONTINUATION OF THE TRIAL BY THE SPONSOR

The Sponsor reserves the right to discontinue this trial at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. The Sponsor shall take advice from the Trial Steering Committee and Data Monitoring Committee as appropriate in making this decision.

11.6 STATEMENT OF CONFIDENTIALITY

Individual participant medical information obtained as a result of this study are considered confidential and disclosure to third parties is prohibited with the exceptions noted above.
Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files.

Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare.

Data generated as a result of this trial will be available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local R&D Departments and the regulatory authorities.

12 USER AND PUBLIC INVOLVEMENT

The Stroke Person's Involvement Group (SPIG), comprising patients and carers ('service users'), have previous supported and helped design research in this area, specifically for the recently completed RECAST-1&2 trials. Jonathan Webb (Stroke 'Conqueror' and member of Royal Derby Hospital Foundation Trust Stroke Operational Group and SPIG) has agreed to join the trial in its design, management, reporting and dissemination. Specifically, he has read and commented on the application, lay summary, issues of capacity and consent, and contributed suggestions as to their improvement. Jonathan Webb will also sit on the Trial Steering Committee as he did for RECAST-1. Lay Summaries - we will develop these in consultation with JW (PPI co-applicant) and the University of Nottingham to ensure summaries are available to participants and are easy to understand, through the trial website. A summary of findings will also be posted on the INVOLVE website (http://www.invo.org.uk/) and disseminated through the Patient, Public and Carer Involvement Leads in the 15 UK Clinical Networks.

13 PUBLICATION AND DISSEMINATION

Reporting, dissemination and notification of the results
Trial results will be published in a peer reviewed academic journal. Reporting will be in compliance with CONSORT recommendations. The focus of that article will be to discuss the effectiveness and safety of RIC in ischaemic stroke. When the study is complete summary findings will post on the support group website. Findings will also be presented at conferences such as UK Stroke Forum, European Stroke Conference and World Stroke Congress.

Policy for publication and authorship
The trial results will be published by named members of the trial team, on behalf of the RECAST-3 Trial Collaborative Group. Members of the collaborative group will be listed in the publication, based on contribution. Any secondary publication may be published by named individuals, but with appropriate acknowledgement of the collaborative group.

14 STUDY FINANCES

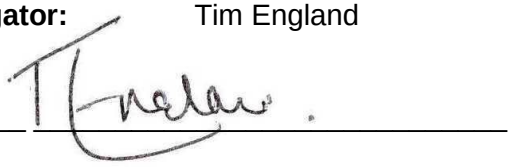
Funding source
Funded by the National Institute of Health Research Efficacy and Mechanism Evaluation (NIHR EME)

Participant stipends and payments
Participants will not be paid to participate in the trial. No additional travel for the trial is expected.

15 SIGNATURE PAGES

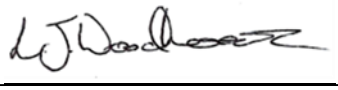
Signatories to Protocol:

Chief Investigator: Tim England

Signature:_____ 

Date: ___23/8/24_____

Trial Statistician: (name)_Lisa Woodhouse____

Signature:_____ 

Date: _27/08/2024_____

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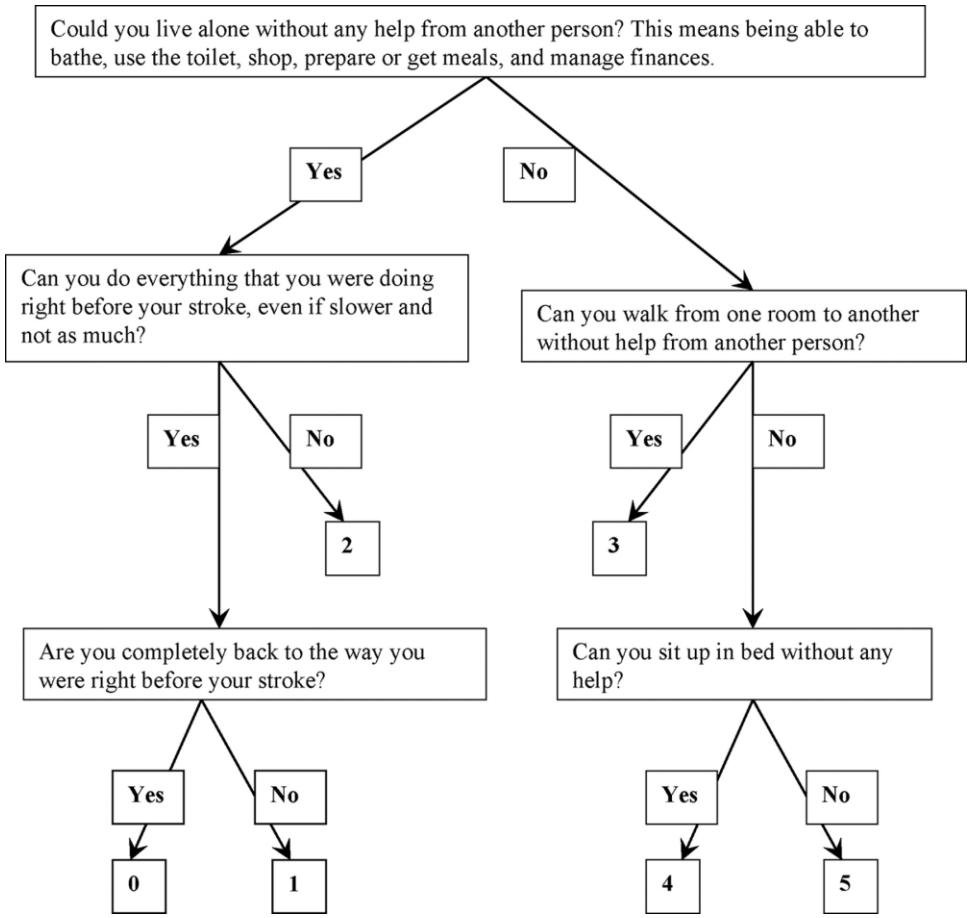
17 Appendices

17.1 Appendix A: modified Rankin Scale

0 No symptoms at all
1 No significant disability, despite symptoms; able to carry out all usual duties and activities
2 Slight disability; unable to carry out all previous activities but able to look after own affairs without assistance
3 Moderate disability; requiring some help, but able to walk without assistance
4 Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5 Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6 Dead

Score 0 to 6 (range 0-6)

Administered via the telephone, as on previous large stroke trials, and validated for use over the phone. The chart below is followed at day 90:[74]



17.2 Appendix B: Outcome event definitions

Cerebrovascular events encompass the following composite of 6 outcomes:

i. **Symptomatic intracranial haemorrhage**: significant neurological deterioration accompanied by clear evidence of significant intracranial haemorrhage on the post randomisation scan (or autopsy if done, i.e. if not rescanned and death occurs). Significant haemorrhage determined if the expert reader both noted the presence of significant haemorrhagic transformation of the infarct or parenchymal haematoma and indicates that haemorrhage is a major component of the lesion (or is remote from the lesion and likely to have contributed significantly to the burden of brain damage). This includes clinical events described as a recurrent stroke, in which the recurrent stroke is confirmed to be caused by an intracranial haemorrhage. Intracranial haemorrhage defined using the Heidelberg Bleeding Classification.[60]

ii. **Symptomatic swelling of the original infarct**: significant neurological deterioration accompanied by evidence of significant brain swelling as determined by the independent masked expert assessment of the scan defined as: shift of the midline away from the side of the ventricle or effacement of the basal cisterns or uncal herniation on a post randomisation scan (or autopsy, if done, i.e. if not rescanned before death). Occurred in 3.5% of the IST-3 population.[6] The presence of some degree of haemorrhagic transformation is permitted, provided it is not identified by the expert CT reader to be a major contributor to the mass effect.

iii. **Extension of ischaemic stroke**: new clinical stroke syndrome judged to be in the same vascular territory as the index event, not attributable to haemorrhage, occurring within the first 72 hours of randomisation. Note, it is clinically and radiologically challenging to differentiate extension of the volume of the original infarct from recurrent embolisation in the same vascular territory. Time-based definition therefore used as in TARDIS.[7]

iv. **Recurrent ischaemic stroke:** new clinical stroke syndrome judged to be in the same vascular territory as the index event, not attributable to haemorrhage, occurring after the first 72 hours of randomisation; or a new clinical stroke syndrome in a different vascular territory to the index event (which can occur at any time point).

v. **Recurrent stroke of unknown type**: new clinical stroke syndrome with no intracranial imaging to determine aetiology

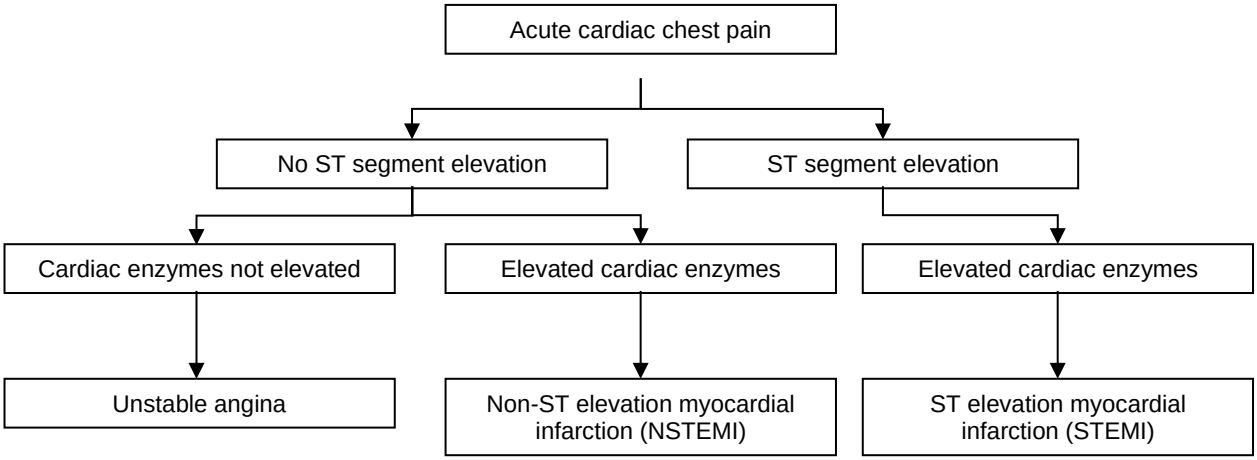
vi. **Neurological deterioration:** an increase in NIHSS score by 4 points or more than the baseline value, not due to cerebral swelling, haemorrhage, recurrent stroke or other recognised cause of decline (e.g. sepsis).

Major adverse cardiac and cerebral events (MACCE) will include: cardiovascular death, MI and all cerebrovascular events (as above)

Myocardial infarction
Acute, evolving or recent MI:[75] (1) Typical rise and gradual fall (troponin) or more rapid rise and fall (CK-MB) of biochemical markers of myocardial necrosis with at least one of the following: (a) ischaemic symptoms; (b) development of pathologic Q waves on the ECG; (c) ECG changes indicative of ischemia (ST segment elevation or depression); or (d) coronary artery intervention (e.g. coronary angioplasty). (2) Pathological findings of an acute MI.

Unstable Angina
Although there is no universally accepted definition of unstable angina, it has been described as a clinical syndrome between stable angina and acute myocardial infarction.

The diagram below will help distinguish between the types of acute coronary syndromes in patients presenting with acute cardiac chest pain:



Acute Kidney Injury

Based on the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline for Acute Kidney Injury (AKI).[76] AKI is defined as any of the following: (i) Increase in serum creatinine (SCr) by ≥0.3 mg/dl (≥26.5 µmol/l) within 48 hours; or (ii) Increase in SCr to X1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; (iii) Urine volume <0.5 ml/kg/h for 6 hours. Grading applied as AKI stage 1-3.

Stage	Serum creatinine	Urine output
1	1.5–1.9 times baseline OR ⩾0.3 mg/dl (⩾26.5 µmol/l) increase	<0.5 ml/kg/h for 6–12 hours
2	2.0–2.9 times baseline	<0.5 ml/kg/h for ⩾12 hours
3	3.0 times baseline OR Increase in serum creatinine to ⩾4.0 mg/dl (⩾353.6 µmol/l) OR Initiation of renal replacement therapy OR, In patients <18 years, decrease in eGFR to <35 ml/min per 1.73 m^2	<0.3 ml/kg/h for ⩾24 hours OR Anuria for ⩾12 hours

17.3 Appendix C: Barthel Index

Task	Criteria	Score
Bowels	Incontinent	0
	Occasional accident (once per week)	5
	Continent	10
Bladder	Incontinent, or catheterised and unable to manage alone	0
	Occasional accident (maximum once per 24 hours)	5
	Continent	10
Grooming	Needs help with personal care	0
	Independent face/hair/teeth/shaving (implements provided)	5
Toilet use	Dependent	0
	Needs some help, but can do something alone	5
	Independent (on and off, dressing, wiping)	10
Feeding	Unable	0
	Needs help cutting, spreading butter, etc.	5
	Independent	10
Transfer (bed to chair and back)	Unable, no sitting balance	0
	Major help (one or two people, physical), cab sit	5
	Minor help (verbal or physical)	10
	Independent	15
Mobility	Immobile	0
	Wheelchair independent, including corners	5
	Walks with help of one person (verbal or physical)	10
	Independent (but may use any aid: for example stick)	15
Dressing	Dependent	0
	Needs help but can do about half unaided	5
	Independent (including buttons, zips, laces, etc.)	10
Stairs	Unable	0
	Needs help (verbal, physical, carrying aid)	5
	Independent	10
Bathing	Dependent	0
	Independent (or in shower)	5

Score out of 100 (range 0-100)

17.4 Appendix D: Functional Oral Intake Scale

1 No oral intake
2 Tube dependent with minimal/inconsistent oral intake
3 Tube supplements with consistent oral intake
4 Total oral intake of a single consistency
5 Total oral intake of multiple consistencies requiring special preparation
6 Total oral intake with no special preparation, but must avoid specific foods or liquid items
7 Total oral intake with no restrictions

17.5 Appendix E: Telephone Cognition

Telephone Mini Mental State Examination and Cognition Scale
Which country are you living in?
Which city/town are you living in?
Which district are you living in?
Which hospital were you admitted to?
Which hospital ward were you on?

"I'm going to give you three words and I'd like you to repeat after me: Apple, Table, Coin". After they have repeated the words successfully, say "Try to remember those because I'm going to ask you for them again later"

Ask the participant, "Can you spell the word 'world' backwards for me?" Note: 'World' should be spelled forward (and corrected) prior to spelling it backwards.

Ask the participant, "Please take 7 away from 100. Now continue to take 7 away from what you have left over until I ask you to stop." Record each answer. If the subject makes a mistake, carry on and check the subsequent answer (e.g. for 93, 84, 77, 70, 63 the score would be 4/5).

Ask the participant if they can remember the three words given earlier?

Ask the participant to repeat “No ifs ands or buts”

What is the thing called that you are speaking into as you talk to me?

What is the time of day?
What day of the week is it?
What is today's date?
What is the month?
What is the year?
What season are we in?
What is your age?
What is your telephone number (code and number)?

Say to the participant, "I am going to read you a list of 10 words. Please listen carefully and try to remember them. When I am done, tell me as many as you can in any order"
[Cabin Pipe Elephant Chest Silk Theatre Watch Whip Pillow Giant]

Please count backwards from 20 to 1

What do people usually use to cut paper?

What is the prickly green plant found in the desert?

What is the name of the reigning monarch (or head of state)?

What is the surname of the current Prime Minister?

What is the opposite direction to East?

Please say this: "Methodist Episcopal"

Ask the participant if they can remember the 10 words given earlier

"I'd like you to name as many animals as possible — any kind of animal. You have one minute.

17.6 Appendix F Zung Depression Scale

Answers are one of:

Seldom or never / Some of the time / Good part of the time / Most of the time

I feel down-hearted and blue

I have trouble sleeping at night

Morning is when I feel best

I can eat as much as I used to

I get tired for no reason

I find it difficult to make decision

I feel hopeful about the future

I feel that I am useful and needed

My life is somewhat empty

I still enjoy the things I used to do

Short Zung IDS Index = 100 x Total / 40
Depression ≥ 70

17.7 Appendix G: Clinical Frailty Scale

Choose one of:

1 Very Fit
People who are robust, active, energetic and motivated. These people commonly exercise regularly. They are among the fittest for their age.

2 Well
People who have no active disease symptoms but are less fit than category 1. Often, they exercise or are very active occasionally, e.g. seasonally.

3 Managing Well
People whose medical problems are well controlled, but are not regularly active beyond routine walking.

4 Vulnerable
While not dependent on others for daily help, often symptoms limit activities. A common complaint is being “slowed up”, and/or being tired during the day.

5 Mildly Frail
These people often have more evident slowing, and need help in high order IADLs (finances, transportation, heavy housework, medications). Typically, mild frailty progressively impairs shopping and walking outside alone, meal preparation and housework.

6 Moderately Frail
People need help with all outside activities and with keeping house. Inside, they often have problems with stairs and need help with bathing and might need minimal assistance (cuing, standby) with dressing.

7 Severely Frail
Completely dependent for personal care, from whatever cause (physical or cognitive). Even so, they seem stable and not at high risk of dying (within ~ 6 months).

8 Very Severely Frail
Completely dependent, approaching the end of life. Typically, they could not recover even from a minor illness.

9.Terminally Ill
Approaching the end of life. This Participating Sites:
category applies to people with a life expectancy <6 months, who are not otherwise evidently frail.

17.8 Appendix H: EuroQOL (EQ-5D-5L)

Group 1
I have no problems in walking about
I have slight problems in walking about
I have moderate problems in walking about
I Have severe problems in walking about
I am unable to walk about

Group 2
I have no problems washing or dressing myself
I have slight problems washing or dressing myself
I have moderate problems washing or dressing myself
I have severe problems washing or dressing myself
I am unable to wash or dress myself

Group 3
I have no problems doing my usual activities (e.g. work, study, housework, family or leisure activities)
I have slight problems doing my usual activities
I have moderate problems doing my usual activities
I have severe problems doing my usual activities
I am unable to do my usual activities

Group 4
I have no pain or discomfort
I have slight pain or discomfort
I have moderate pain or discomfort
I have severe pain or discomfort
I have extreme pain or discomfort

Group 5
I am not anxious or depressed
I am slightly anxious or depressed
I am moderately anxious or depressed
I am severely anxious or depressed
I am extremely anxious or depressed

Health state today by visual analogue scale (best imaginable to worst imaginable)