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

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Acronym: *RECAST-3*

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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	Dr Tim England
Aim	To perform a multicentre randomised controlled trial assessing remote ischaemic conditioning (RIC) in patients with hyperacute ischaemic stroke
Trial Configuration	Phase III prospective randomised (1:1) sham-controlled blinded-endpoint parallel-group multicentre trial.
Setting	Adults with hyperacute 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:** Hyperacute ischaemic stroke (≤6 hours post onset); primary intracerebral haemorrhage ruled out on baseline clinical neuroimaging; NIHSS score ≥4 at randomisation; age ≥18 years **Exclusion criteria:** Pre-morbid dependency (modified Rankin Scale, mRS>3); Spontaneous intracerebral haemorrhage; Dementia; Coma (GCS <8)); Malignancy; Significant co-morbidity (life expectancy <6 months); BM <3.0mmol/L; Seizure on presentation unless brain imaging identifies evidence of significant brain ischaemia.
Description of interventions	**Intervention:** RIC group: 4 cycles of intermittent limb ischaemia - alternating 5 minutes inflation (+20 mmHg above systolic BP) followed by 5 minutes deflation of an automated upper arm blood pressure cuff. **Comparator:** Sham RIC. An automated upper arm blood pressure cuff is inflated to 20 mmHg for 4 cycles (5minutes inflation/5 minutes deflation). **Duration of treatment:** First dose (4 cycles of RIC or sham) within <6 hours of onset. Second dose 1-2 hours after the first dose. Twice daily until end day 2; total 4 doses.
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; COVID-19 status; disability; cognition; mood; frailty; quality of life; safety (death; neurological deterioration; intracranial haemorrhage, systemic embolism, serious adverse events) **Mechanisms**: Ischaemic reperfusion injury (Day 2 CT brain: intracranial haemorrhagic, swelling of original stroke, recurrent ischaemic stroke); mechanical thrombectomy substudy (Day 7 MRI; infarct growth and volume, oedema, perfusion).

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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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, 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, to a level exceeding the systolic blood pressure for 5 minutes in order to induce ischaemia in the limb, 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.

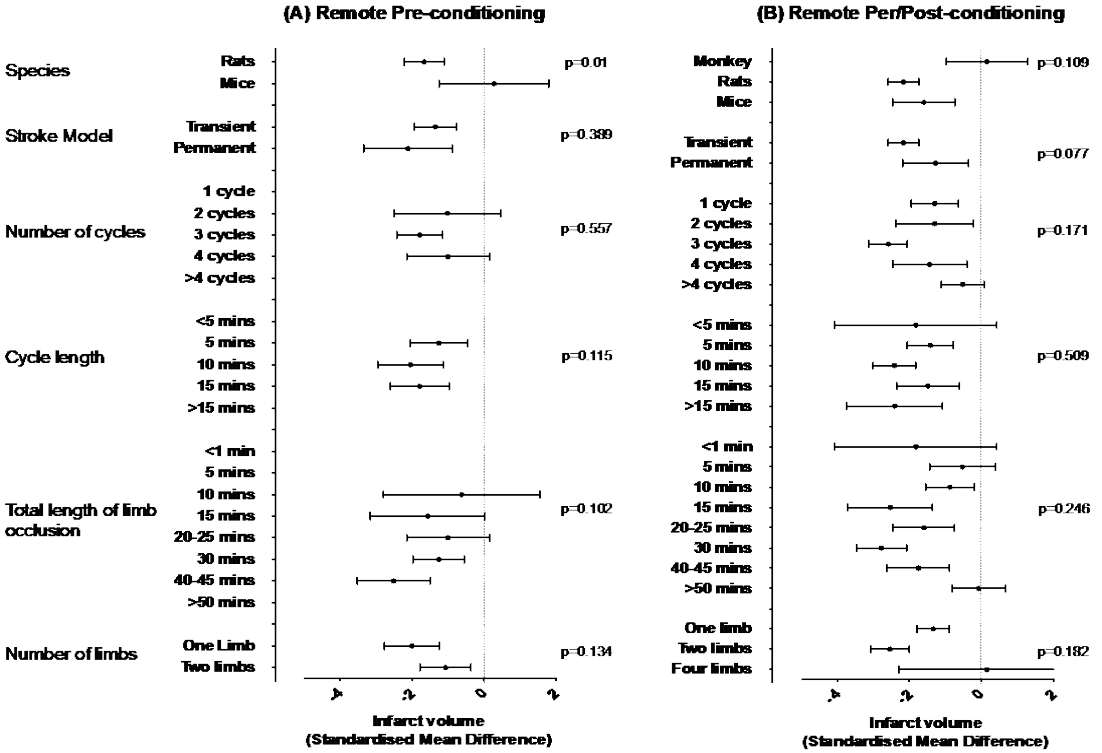
Following our pilot and feasibility studies, RECAST-1 & 2, we propose to perform a clinical phase III efficacy randomised controlled trial of RIC in hyperacute stroke across multiple UK sites. The trial is also designed to address mechanisms of action including testing the effect of RIC on clinical and radiological 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



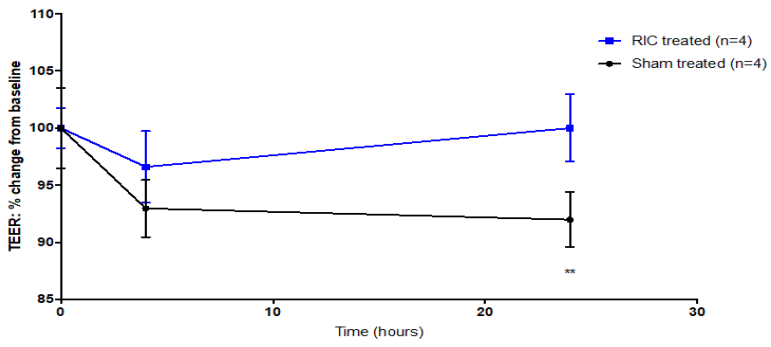
Clinical trials

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 perconditioned 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.

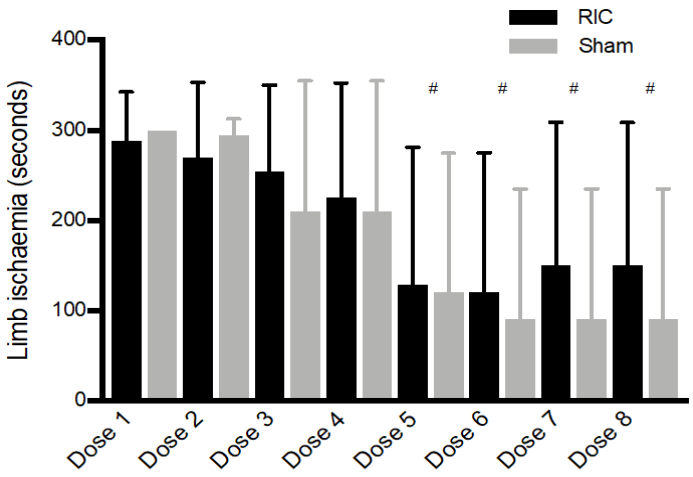
RECAST-1 (CI England, n=26) demonstrated excellent intervention tolerability using one dose of 4 cycles of upper limb ischaemia and reperfusion (5min/5min) performed with 24 hours of stroke, excluding those thrombolysed.[24] Although limited by a small sample size, there was a significant decrease in 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.

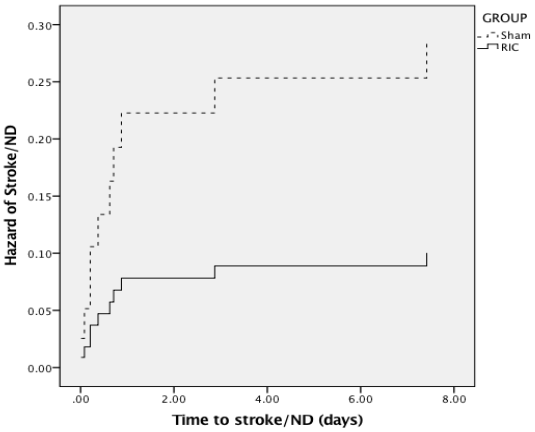
Figure 3. Mean (±SD) time of RIC adherence in RECAST-2. One dose = 4 cycles (5min/5min) of upper limb ischaemia/reperfusion



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.

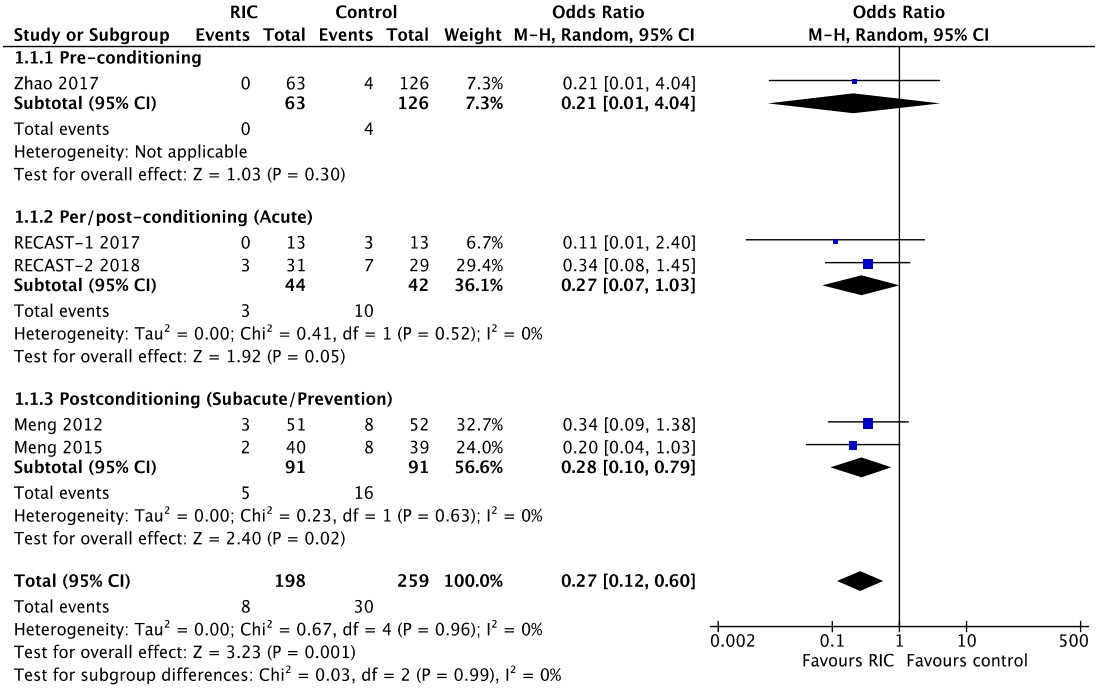
Figure 4. RECAST-2: risk of recurrent stroke and neurological deterioration (fatal and non-fatal) by RIC or sham



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 stroke 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, yet to start) 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) is selecting 200 participants using MRI, applying RIC to the leg. REPOST (Netherlands Trial Register, NTR6880) is using twice daily upper limb RIC for 4 days in AIS, started within 12 hours of onset (probably too late). Similarly, the RICAMIS trial (NCT03740971, planned n=1800, China) has recently been registered, which will perform upper limb RIC within 48 hours of ischaemic stroke. 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) is using RIC daily for 300 days in symptomatic intracranial arterial stenosis. RIC trials in post-stroke fatigue (NCT03794947) and motor recovery in chronic stroke (NCT03095755) are registered but not directly relevant to this application.

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) [35, 36], reasons for this included the potential interaction with the anaesthetic agent Propofol [37], 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 perconditioning: 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. [38, 39]). A systematic review of these studies suggests the significant reduction in myocardial damage may not be clinically meaningful [40]. 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 [41]; 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 [42]. 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 was
published recently [43] and 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 [44] and drugs modulating opioid receptors [45]. 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: (i) Exclusion criteria: Importantly, RECAST-3 already excludes patients on long-term nitrate therapy and patients with diabetes mellitus (another factor recognised to diminish RIC efficacy [46]; 11% had medically controlled diabetes in CONDI-2/ERIC-PPCI); (ii) In addition to treating a different organ, other key differences include the use of repeated RIC dosing over 2 days as used in RECAST-2 [28] (compared to a single 'dose' in CONDI-2/ERIC-PPCI) and (iii) 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 a well designed phase III trial in hyperacute stroke.

DETAILS OF INVESTIGATIONAL MEDICAL DEVICE

Device Description

Developed with Dr R Blauenfeldt (Dept of Neurology, Aarhus University, Denmark) and Seagull Healthcare (Herlufmagle, Denmark), originally for the RESIST trial. The cuff is placed on the upper arm and activated by pressing a single start button on the RIC device (pictured below); a blood pressure is measured, then the device inflates to +20 mmHg above the systolic reading and maintains the pressure for 5 minutes followed by 5 minutes deflation (arm reperfusion); 4 cycles are completed automatically.

The device is based on a standard CE marked BP monitor manufactured by Shenzhen Raycome Health Technology Co Ltd. Timestamps, BP, cuff pressure and total RIC cycles are recorded and stored, providing compliance data.

The device has been modified from its CE marked version as detailed in the Technical Dossier. The manufacturer has no intention to alter the CE mark, distribution or marketing authorisation of the product at this point. Due to the modification and use in a multi-centre trial a letter of no objection from the MHRA will be sought as required under the UK Medical Devices regulations, SI 2002, No. 618 (as amended).

A separate sham device matching exactly in appearance inflates only to 20 mmHg during RIC.

Please refer to the separate Technical Dossier for details.

Packaging and labelling

Device arrives in displayed packaging

Display when device switched on

On/off switch

USB charging point

Set/Val buttons – adjusts time/date

Start button – press once to open display

Display after start button is pressed and the initial BP is taken

Battery life

Time

Systolic BP

Diastolic BP

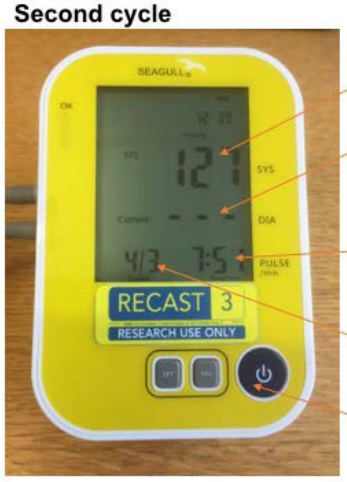
Pulse

Start button – Press to take initial BP. The device will then continue to deliver 4x RIC/sham cycles automatically

Display during first and second cycles

First cycle

Second cycle



Original systolic reading

Current cuff pressure is obscured to maintain blinding

Time remaining in cycle
(cuff is inflated during first 5 minutes, deflated in the next 5 minutes)

Total Nº of cycles / Nº of cycles remaining

Stop button – will stop the cycles and rapidly deflate the cuff if pressed during the cycle

Control Devices

RIC Device

Sham Device

RIC and Sham Device are identical except for labels on the underside of the device

Storage, supply and return

The trial management team will be responsible for suppling the devices, which have been purchased from Seagull Healthcare. It is likely that each centre will require one RIC device and one sham device only, though high recruiting centres may require additional devices. Should centres wish to take part and no further devices are available, application of RIC or sham can be applied using a manual sphygmomanometer as in RECAST-1 & RECAST-2. When not in use, the RIC and Sham Devices should be held in a securely locked cupboard, only accessible to authorised personnel. Redundant devices can be returned to the coordinating centre in Nottingham.

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, 38] 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 trial, which recruited 5400 patients trial in an unscreened population with acute myocardial infarction and paramedic RIC administration.[43] Unexpected adverse events will be reported to Nottingham Stroke Trials Unit. Confirmed unexpected SAEs will be notified to the Sponsor, MHRA, 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 (including the sham device) will be accounted for by the investigator using device accountability forms.

TRIAL OBJECTIVES AND PURPOSE

PURPOSE

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

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

PRIMARY OBJECTIVE

Primary research question: Does RIC improve functional outcome (ordinal shift in mRS) at day 90 in patients with hyperacute ischaemic stroke?

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? (Day 2 CT brain, MT sub study)

TRIAL DESIGN

TRIAL CONFIGURATION

Design: Prospective randomised sham-controlled blinded-endpoint parallel-group multicentre trial of RIC versus control. 1,300 patients with hyperacute (<6 hours) 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.[47]

Secondary outcomes

Clinical (day 90):
Cerebrovascular events by day 90;[†] mRS (binary);[47] major adverse cardiac and cerebrovascular events (MACCE: cardiovascular death, MI, all stroke); AKI;[48] COVID-19 status; disability (Barthel Index, BI); cognition (TICS-M); mood (Zung Depression Scale); Frailty (Clinical Frailty Scale, CFS);[49] Quality of Life (EQ-5D-5L); home-time;[7, 8] recorded with mRS via telephone.

Compliance: recorded by automated device**.**

Safety endpoints

Safety (day 2, 4 & 90)**:** death; recurrent IS, intracranial haemorrhage, symptomatic swelling of the original infarct;[6] neurological deterioration; transient ischaemic attack (TIA); systemic embolism, neurovascular limb compromise.

Other SAEs >1 week will not be collected; thereafter, only fatal SAEs and outcomes will be recorded and blindly adjudicated.

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.[50]

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 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:[51] (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.

Acute Kidney Injury

Based on the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline for Acute Kidney Injury (AKI). AKI is defined as any of the following: (i) Increase in serum creatinine (SCr) by x0.3 mg/dl (x26.5 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.

COVID-19

Due to the global negative impact of the COVID-19 pandemic on healthcare and the unknown consequences on ischaemic stroke treatment and recovery, the presence or absence of COVID-19 infection will be collected at baseline (confirmed through swab or blood testing, or clinically suspected) and at follow up by day 90.

Mechanistic studies

Neuroimaging

Putative anti-platelet effects of RIC could increase risk of intracranial haemorrhage (including haemorrhagic transformation of infarction, intracerebral haemorrhage, intraventricular haemorrhage), especially post-thrombolysis. Day 2 CT brain (all participants) will also assess for evidence of reperfusion injury: swelling of original infarct and intracranial haemorrhage (Heidelberg bleeding classification[50]). Imaging based asymptomatic events (no significant neurological deterioration) will also be counted.

Sub-study: Mechanical thrombectomy, MT (n=50).

Participants in this mechanical thrombectomy sub-study will all receive a standard of care MRI brain scan. A research MRI will be performed at day 7 assessing the pleiotropic effects of RIC:

(i) Infarct volume - Day 7 FLAIR volume at 1 week, which correlates significantly with final infarct volume (correlation coefficient 0.93);[52] (

ii) Infarct growth: Day 7 MR FLAIR stroke volume - Day 1 DWI.[53]

(iii) Cerebral oedema: (∂DWI) using region of interest analysis, we will partition swelling from infarct volume growth,[54] 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;[55]

(v) Haemorrhagic transformation of infarction, HTI (T2*-weighted imaging or SWI)

Initially, this substudy will only be performed at University College London (UCL) where MRI at day 1 is performed as standard of care. If other centres express a wish to take part, this will be considered depending on MRI availability and the development/standardisation of the MRI protocols.

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, BP, sex, time since stroke, stroke severity (NIHSS) and COVID-19 status (confirmed or suspected).

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. The choice of +20mmHg above the systolic blood pressure in the treatment arm is selected to enhance patient comfort and tolerance compared to inflating to >200mmHg, which is 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.[56]

Maintenance of randomisation codes and procedures for breaking code

In general there should be no need to unblind the allocated treatment since it is a one-off procedure. Unblinding 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.

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 (SDMC)
An independent chair will run the SDMC with 2 other independent members. Unblinded data provided by Nottingham CTU statisticians; meetings planned biannually. Interim analysis performed at 50% recruitment with 90 day follow up. The Chief Investigator and the SDMC can request more meetings if deemed necessary for safety.

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 18 months. Assuming the success criteria have been met, this will run seamlessly (i.e. without halting recruitment) in to the main phase (phase 2) of the trial.

Stop-go decision
The trial will proceed to the main phase at 18 months if >85% of the vanguard phase participants have been recruited (n>306 of anticipated 360). If 50-85% of target is reached at 18 months, we will review strategies to improve recruitment/follow up and proceed with further monitoring as agreed with the TSC. If <50% of target, and all strategies to improve recruitment/retention have been implemented but not resulted in improvement, the TSC will terminate the study.

Measurable recruitment objectives
The measurable objectives below are a guide but we will open sites as soon as is practically feasible and aim to reach 60 sites to reach optimal recruitment as soon as possible.

- 3 months: First Participant
- 6 months: 15-20 active sites, 45 participants recruited
- 12 months: 30 active sites, 175 participants recruited
- 18 months: 40 active sites, and 360 participants recruited.
  Stop/go decision at 18 months: trial will continue if ≥85% recruitment achieved (see detail in 5.17.1)
- DMC interim analysis at 18 months (~300 participants followed up for 90 days)
- 24 months: 60 active sites, and 600 participants recruited
- 30 months: 60 active sites, and 950 participants recruited
- 36 months: 60 active sites and 1300 participants recruited

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

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.

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

Inclusion criteria:

1) Hyperacute ischaemic stroke ($\leq$6 hours post onset);
2) Spontaneous intracerebral haemorrhage ruled out on baseline clinical neuroimaging; Haemorrhagic transformation of infarction (HTI) HI1, HI2, PH1[50] is permitted.
3) NIHSS score $\geq$4 at randomisation;
4) Age $\geq$18 years

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) Spontaneous intracranial haemorrhage; potential RIC antiplatelet effect could exacerbate this.
3) Haemorrhagic transformation of infarction PH2 (haematoma occupying 30% or more of the infarcted tissue, with obvious mass effect)
4) Dementia,
5) Coma (GCS <8),
6) Malignancy, and significant co-morbidity (life expectancy <6 months): factors that will lead to a poor outcome, no matter the intervention
7) Capillary blood glucose <3.0mmol/L; hypoglycaemia sufficient to account for neurological symptoms.
8) 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.
9) 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.
10) Known pregnancy – whilst RIC is not expected to be harmful, there are no data currently to support this. "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.''

Note: We have considered excluding participants with known presence of subclavian or upper limb arterial stenosis but there were no adverse events relating to this in CONDI2/ERIC-PPCI (n=5400), an unscreened population with acute myocardial infarction and paramedic RIC administration.

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.

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 [57] 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:

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 maximum of 15 minutes should be taken obtaining consent. 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.

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 a blood pressure cuff) 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**. 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.

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:

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 a blood pressure cuff) 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 cuff applied to their arm for 40 minutes whilst the rest of their usual treatment continues. 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.

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 patients 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 a blood pressure cuff) 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 cuff applied to their arm for 40 minutes whilst the rest of their usual treatment continues. If they say yes, the potential participant will be randomised **using this initial oral consent.** Full Consultee Advice will be obtained as soon as practicable. Full informed written consent will be obtained from the patient if capacity is regained.

Relatives not present: If the patient lacks capacity and no consultee is present, we will not recruit the patient into the 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). 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.

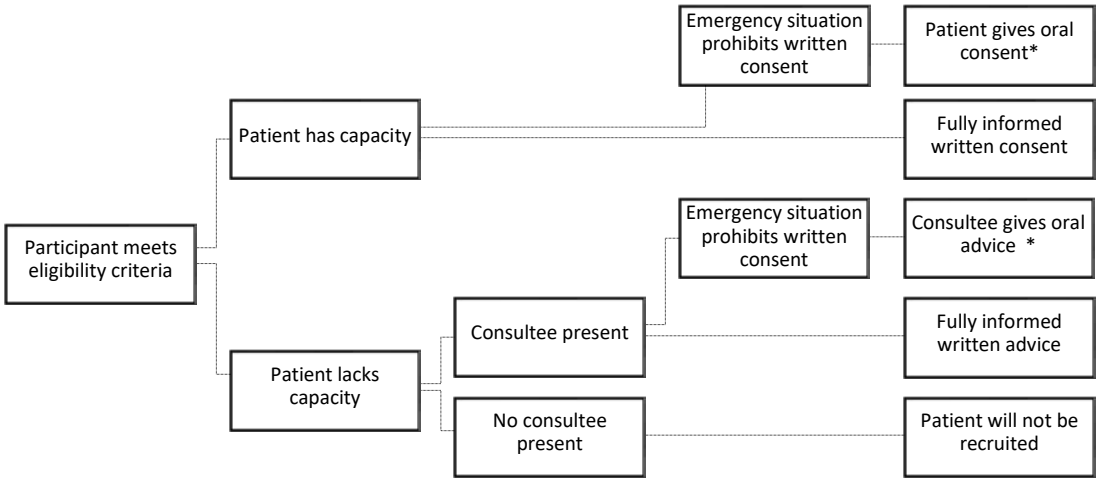
Where the patient is being assessed and treated via telemedicine (as is often standard care in many stroke services out of hours) by a member of the medical team who is appropriately trained and listed on the delegation log, the process is as above, with the exception that the paper consent form will 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).

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) compliance/adherence data from our dose escalation trial RECAST-2.[28]

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 automated cuff will be placed ideally on the non-paretic upper arm to deliver RIC or sham (the other arm can be used if the non-paretic arm is needed for clinical reasons). The device is programmed to cycle 4 times (=1 dose), and 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 an automated upper arm blood pressure cuff.
- **Control:** Sham RIC. An automated upper arm blood pressure cuff is inflated to 20 mmHg for 4 cycles (5minutes inflation/5 minutes deflation).

The sham was feasible in RECAST-2 since when asked at day 90 which intervention they received, 56 (93%) participants did not know, 2 (4%) were incorrect and 2 (4%) correct.

Duration of treatment:

- First dose (4 cycles of RIC or sham) within <6 hours of onset.
- Second dose 1-2 hours after the first dose.
- Twice daily until end day 2; total 4 doses (compliance drops to 40% on day 3, Figure 3 in background).[28]

Automated Device

The RIC device cuff is placed on the upper arm and activated by pressing a single start button; a blood pressure is measured, then the device inflates to +20 mmHg above the systolic reading and maintains the pressure for 5 minutes followed by 5 minutes deflation (arm reperfusion); 4 cycles are completed automatically. A matching separate sham device inflates only to 20 mmHg.

In the unlikely event of a device failure, or it is unavailable (e.g. due to use for another participant), the RIC or sham procedure can be carried out using a manual sphygmomanometer, as performed in RECAST-1 and RECAST-2. The attending investigator can manually inflate the BP cuff on the upper arm to the desired pressures as per the cycles described above). The investigator can use the device once available for any repeated doses if necessary.

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:

Follow-up

Day 1
Following consent and randomisation, baseline routine clinical assessments, including pre-intervention BP, will be recorded from the medical notes. The patient will then receive RIC or placebo using the automated device. If the device is not available, RIC or placebo can be administered manually using a manual sphygmomanometer. A blood pressure after RIC/placebo is taken. A second dose of 4 cycles is applied 1-2 hours after the end of the first dose.

Day 2
Two further doses of RIC or sham are applied, once in the morning and once in the afternoon. A second CT brain is performed and a further neurological (NIHSS) and safety assessment. The CT may be part of routine care (e.g. post thrombolysis scan) and can be used for trial purposes. All participants require the CT brain on day 2 for assessments of safety and reperfusion injury.

Day 4 (±1) (or on discharge if earlier)
Clinical assessment is performed including (NIHSS and safety (e.g. new outcome events). This will be performed in hospital.

Day 7 (±2) (selected sites only)
Only participants in the mechanical thrombectomy MRI substudy will have a MRI at Day 7 (±2). (Local sites will need to arrange return for the day 7 scan if the participant has already been discharged)

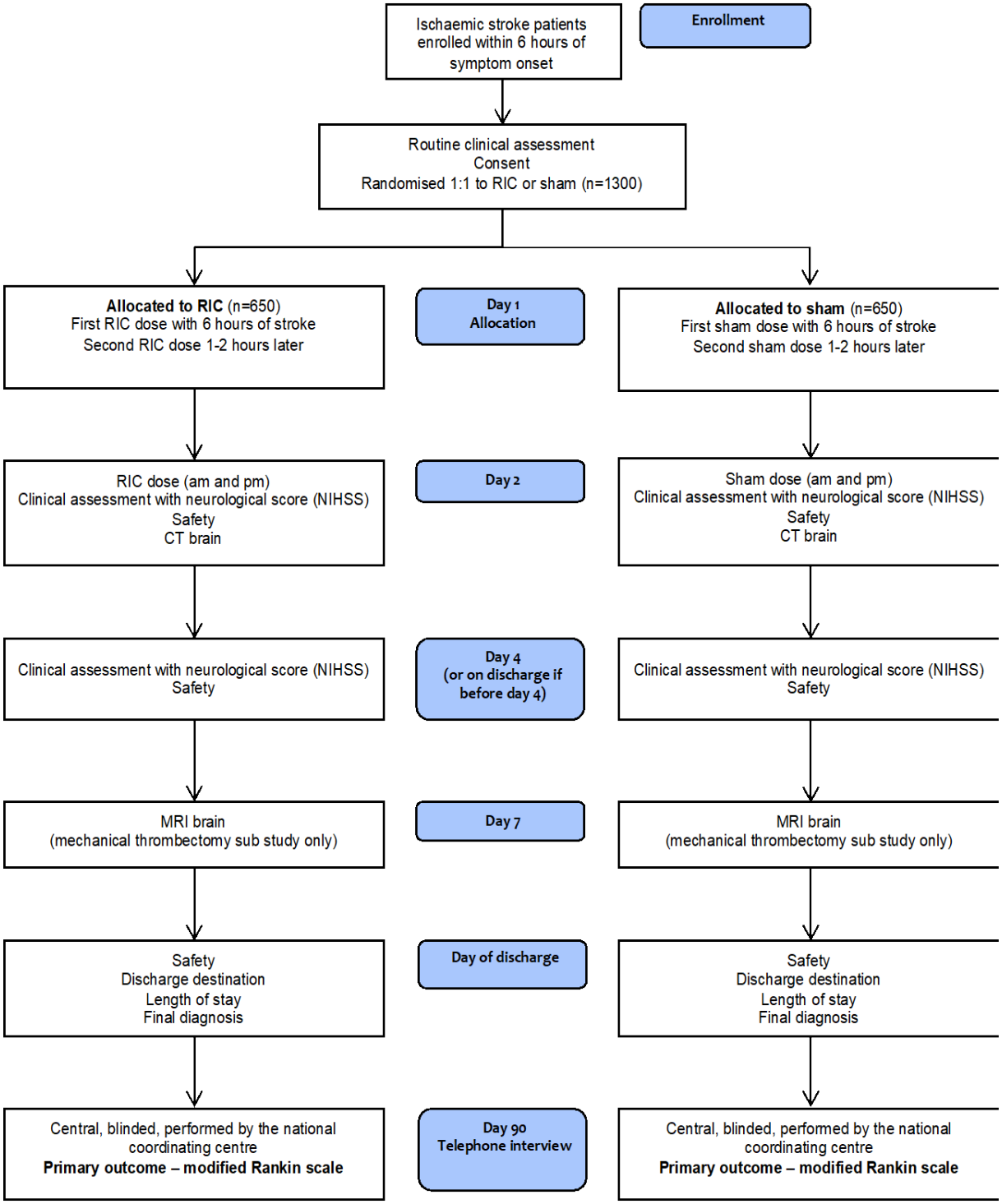
Discharge or death
Information provided on final diagnosis, length of stay, discharge destination, clinical scans for stroke phenotyping, and secondary outcome data collection

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	**Admission**	**1**	**2**	**4**	**7**	**90**
All patients:						
CT head scan	X *		X			
Consent		X				
RIC /sham		X X†	X X††			
Clinical efficacy:						
Impairment: (NIHSS)		X *	X	X		
Day 90 outcomes via telephone						X
Safety		X	X	X		X
MRI brain (selected centres only)					X	

RIC, remote ischaemic conditioning; NIHSS, National Institutes of Health Stoke Scale; * Performed as part of routine clinical care.

RECAST-3 Trial Flow



Remote ischaemic conditioning (RIC): An automated upper arm blood pressure cuff is inflated to +20 mmHg above the systolic BP for 4 cycles (5 minutes inflation & 5 minutes deflation).
Sham: An automated upper arm blood pressure cuff is inflated to 20 mmHg for 4 cycles (5 minutes inflation & 5 minutes deflation).
RIC/sham performed within 6 hours of stroke onset; repeated after 1-2 hours, then bd on Day 2. A total of 4 doses.

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 include 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; Day 4 (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. All trial participants will have an additional CT scan 24 hours (± 6 hours) after randomisation (a safety measure due to the putative antiplatelet effects of RIC) to assess for evidence of reperfusion injury. 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 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 7 MRI (from MT substudy) 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 cuff is tolerated and the number of cycles completed. The automated device 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 placebo.

RADIATION EXPOSURE

Details of diagnostic or therapeutic ionising radiation

Participation will receive a routine clinical non-contrast single run CT head scan at the time of presentation with stroke and an additional non-contrast single run CT head scan at the end of the treatment. 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.

Details of radioactive materials and dose

From National Radiological Protection Board –W67 'Doses from computed tomography (CT) examinations in the UK' (2003 review), the doses from CT scans will vary between sites with different models of equipment and between different sizes of patient. A CT of the brain will give an average of 1.5mSv but this could be up to a maximum of 5mSv. So a typical dose from CT due to research exposures would be 1.5mSv, but could be as high as 5mSv. A 1.5mSv dose would be roughly equivalent to 8 months of exposure to natural background radiation to a member of the public resident in the UK. A 5mSv dose is roughly equivalent to 2¼ years of background received by a member of the public resident in the UK.

Risk Assessment (induction of fatal cancer)

Based on a risk coefficient for developing fatal radiation induced cancer (all ages) of 5%/Sv (ICRP), this would lead to a risk of radiation exposure incurred as part of the trial similar to the annual risk of dying from an accident in the home.

This is classed as an intermediate risk and the required benefit should be aimed directly at diagnosis, cure or prevention of disease.

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.

The objective of the exposure is to assess the extent of the bleeding (haematoma) in 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 procedure for CT and any doses in lay terms are explained in the participant information sheet.

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 [58] 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 differ between those participants randomised to RIC versus 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 [59] 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[57]) & covariate adjustment reducing sample size by 20%;[60] 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

In summary, a trial of 1,300 participants (940 from main phase and 360 from start-up phase) will have 90% power to detect an ordinal shift of mRS outcome with odds ratio 0.75.

Sample size: secondary outcomes

Cerebrovascular events: In RECAST-2 (n=60, 55% thrombolysed), the composite of recurrent cerebrovascular events (symptomatic recurrent or extension of infarction, symptomatic intracranial haemorrhage, ND) occurred in 24% of the control group and 10% in the RIC group. These are potentially over/under-estimates in a small sample. In a similar and larger population (IST-3, n=3035, 50% thrombolysed), recurrent cerebrovascular event rate was 17.5% (symptomatic swelling of infarct 3.5%; symptomatic intracranial haemorrhage 4%; ND 9%; recurrent ischaemic

stroke 1%).[6] Assuming a 17.5% event rate in the control group vs 12.5% in the treatment group, power 90%, alpha 0.05, 5% loss to follow up and adjustment for co-variates, 1284 patients would detect a HR of 0.65 (Cox's proportional hazard model, time to first event) in favour of RIC. In RECAST-2, adjusted HR is 0.28 (95%CI 0.06-1.2, p=0.08) but in a small population.

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: 'Shift' in day 90 mRS between treatment groups using ordinal logistic regression (OLR, following a check for proportionality of odds) with adjustment for minimisation variables & other pre-specified prognostic baseline factors.[47]

Interim Analyses. An interim analysis after 18 months (anticipated ~300 participants recruited and followed up for 90 days will be performed by the Data Monitoring Committee. Safety analyses will be performed 6 monthly.

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 (0-2hours, 2-4hours, 4-6hours); 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); COVID status (no/suspected/yes). 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 identified 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. The DMC will perform a formal interim analysis after 18 months recruitment. 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.

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 > 6 hours from onset of symptoms
3. Participant less than 18 years of age
4. Primary intracerebral haemorrhage present on baseline clinical neuroimaging (haemorrhagic transformation of is permitted)
5. NIHSS score <4 at randomisation
6. Dementia
7. Coma (GCS <8),
8. Known probable life expectancy of less than 3 months
9. Capillary blood glucose <3.0mmol/L
10. 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)
11. 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. 7-day follow-up: >7 days past the due date
    c. Hospital event 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: >1day past the due date
b. 4-day follow-up: >2days past the due date
c. Hospital event form: >7days 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.

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 lead 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.[50]
- symptomatic swelling of the original infarct;[6]

- neurological deterioration;
- systemic embolism;
- neurovascular limb compromise secondary to RIC
- myocardial infarction
- AKI

All SAEs up to and including Day 7 will be collected. SAEs >1 week will not be collected; thereafter, only fatal SAEs and outcomes will be recorded and blindly adjudicated.

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 Technical Dossier.

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.

SAEs are very common after stroke but considering the effects of RIC in both ischaemic and haemorrhagic stroke are relatively unknown, all SAEs (to day 90) shall be recorded.

Reporting of adverse events

All adverse events (AEs) will be recorded as they are reported 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. 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 adverse events and adverse device effects will be recorded and reported to the MHRA and REC as part of the annual reports.

SAEs and SADEs will be reported within the statutory timeframes to the MHRA and REC 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.
- If the event is deemed a SAE or SADE, shall, within 7 days, complete the appropriate adverse incident report form available from the MHRA web page and send to the MHRA
- 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 MHRA and 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.

ETHICAL AND REGULATORY ASPECTS

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 Medicines and Healthcare products Regulatory Agency (MHRA), 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 MHRA, 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.

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 investigator or their nominee and the participant or consultee shall both sign and date the 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).

RECORDS

Device accountability

Device supplies will be kept in a secure, limited access storage area 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. 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 made be available at all times for review by the Chief Investigator, Sponsor's designee and inspection by relevant regulatory authorities.

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.

QUALITY ASSURANCE & AUDIT

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, research participants and research protocols with both public liability insurance and clinical trials insurance. These policies include provision for indemnity in the event of a successful litigious claim for proven non-negligent harm.

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.

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.

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.

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.

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.

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 be 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.

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.

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.

SIGNATURE PAGES

Signatories to Protocol:

Chief Investigator: (name)_____

Signature:_____

Date: _____

Trial Statistician: (name)_____

Signature:_____

Date: _____

REFERENCES

1. Saka O, McGuire A, Wolfe C. Cost of stroke in the United Kingdom. *Age Ageing*. 2009;38:27-32
2. Sandercock P, Gubitz G, Foley P, Counsell C. Antiplatelet therapy for acute ischaemic stroke. *Cochrane Database Syst Rev*. 2003:CD000029
3. Wardlaw JM, Murray V, Berge E, Del Zoppo GJ. Thrombolysis for acute ischaemic stroke. *Cochrane Database Syst Rev*. 2009:CD000213
4. Berkhemer OA, Fransen PS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med*. 2015;372:11-20
5. Shuaib A, Lees KR, Lyden P, Grotta J, Davalos A, Davis SM, et al. NXY-059 for the treatment of acute ischemic stroke. *New England Journal of Medicine*. 2007;357:562-571
6. Sandercock P, Wardlaw JM, Lindley RI, Dennis M, Cohen G, Murray G, et al. The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. *Lancet*. 2012;379:2352-2363
7. Bath PM, Woodhouse LJ, Appleton JP, Beridze M, Christensen H, Dineen RA, et al. Antiplatelet therapy with aspirin, clopidogrel, and dipyridamole versus clopidogrel alone or aspirin and dipyridamole in patients with acute cerebral ischaemia (TARDIS): a randomised, open-label, phase 3 superiority trial. *Lancet*. 2018;391:850-859
8. ENOSInvestigators. Efficacy of nitric oxide, with or without continuing antihypertensive treatment, for management of high blood pressure in acute stroke (ENOS): a partial-factorial randomised controlled trial. *Lancet*. 2014
9. Hess DC, Blauenfeldt RA, Andersen G, Hougaard KD, Hoda MN, Ding Y, et al. Remote ischaemic conditioning-a new paradigm of self-protection in the brain. *Nat Rev Neurol*. 2015;11:698-710
10. Hausenloy DJ, Barrabes JA, Botker HE, Davidson SM, Di Lisa F, Downey J, et al. Ischaemic conditioning and targeting reperfusion injury: a 30 year voyage of discovery. *Basic Res Cardiol*. 2016;111:70
11. Li S, Hu X, Zhang M, Zhou F, Lin N, Xia Q, et al. Remote ischemic post-conditioning improves neurological function by AQP4 down-regulation in astrocytes. *Behav Brain Res*. 2015;289:1-8
12. Ma J, Ma Y, Dong B, Bandet MV, Shuaib A, Winship IR. Prevention of the collapse of pial collaterals by remote ischemic perconditioning during acute ischemic stroke. *J Cereb Blood Flow Metab*. 2017;37:3001-3014
13. Zhang Y, Ma L, Ren C, Liu K, Tian X, Wu D, et al. Immediate remote ischemic postconditioning reduces cerebral damage in ischemic stroke mice by enhancing leptomeningeal collateral circulation. *J Cell Physiol*. 2019;234:12637-12645
14. Liu C, Yang J, Zhang C, Geng X, Zhao H. The changes of systemic immune responses during the neuroprotection induced by remote ischemic postconditioning against focal cerebral ischemia in mice. *Neurol Res*. 2019;41:26-36
15. Cheng Z, Li L, Mo X, Zhang L, Xie Y, Guo Q, et al. Non-invasive remote limb ischemic postconditioning protects rats against focal cerebral ischemia by upregulating STAT3 and reducing apoptosis. *Int J Mol Med*. 2014;34:957-966
16. Hu S, Dong H, Zhang H, Wang S, Hou L, Chen S, et al. Noninvasive limb remote ischemic preconditioning contributes neuroprotective effects via activation of adenosine A1 receptor and redox status after transient focal cerebral ischemia in rats. *Brain Res*. 2012;1459:81-90
17. Guo ZN, Guo WT, Liu J, Chang J, Ma H, Zhang P, et al. Changes in cerebral autoregulation and blood biomarkers after remote ischemic preconditioning. *Neurology*. 2019;93:e8-e19
18. Weir P, Maguire R, O'Sullivan SE, England TJ. Remote ischaemic conditioning in experimental stroke: a systematic review and meta-analysis (UK Stroke Forum). *International Journal of Stroke*. 2018;13:48
19. Sun J, Li T, Luan Q, Deng J, Li Y, Li Z, et al. Protective effect of delayed remote limb ischemic postconditioning: role of mitochondrial K(ATP) channels in a rat model of focal cerebral ischemic reperfusion injury. *J Cereb Blood Flow Metab*. 2012
20. Ren C, Wang P, Wang B, Li N, Li W, Zhang C, et al. Limb remote ischemic per-conditioning in combination with post-conditioning reduces brain damage and promotes neuroglobin expression in the rat brain after ischemic stroke. *Restor Neurol Neurosci*. 2015;33:369-379
21. Hoda MN, Siddiqui S, Herberg S, Periyasamy-Thandavan S, Bhatia K, Hafez SS, et al. Remote ischemic perconditioning is effective alone and in combination with intravenous tissue-type plasminogen activator in murine model of embolic stroke. *Stroke*. 2012;43:2794-2799
22. Hildebrandt HA, Kreienkamp V, Gent S, Kahlert P, Heusch G, Kleinbongard P. Kinetics and Signal Activation Properties of Circulating Factor(s) From Healthy Volunteers Undergoing Remote Ischemic Pre-Conditioning. *JACC Basic Transl Sci*. 2016;1:3-13

23. Hougaard KD, Hjort N, Zeidler D, Sorensen L, Norgaard A, Hansen TM, et al. Remote ischemic perconditioning as an adjunct therapy to thrombolysis in patients with acute ischemic stroke: a randomized trial. *Stroke*. 2014;45:159-167
24. England TJ, Hedstrom A, O'Sullivan S, Donnelly R, Barrett DA, Sarmad S, et al. RECAST (Remote Ischemic Conditioning After Stroke Trial): A Pilot Randomized Placebo Controlled Phase II Trial in Acute Ischemic Stroke. *Stroke*. 2017;48:1412-1415
25. Shimada Y, Tanaka R, Shimura H, Yamashiro K, Urabe T, Hattori N. Phosphorylation enhances recombinant HSP27 neuroprotection against focal cerebral ischemia in mice. *Neuroscience*. 2014;278:113-121
26. Stone N, Hedstrom A, O'Sullivan SE, England T. Remote ischaemic per-conditioning provides prolonged neuroprotection 4 days after a single dose: data from an ex-vivo in-vitro blood brain barrier model. *ESO Conference*. 22nd May 2019
27. Hind WH, England TJ, O'Sullivan SE. Cannabidiol protects an in vitro model of the blood-brain barrier from oxygen-glucose deprivation via PPARgamma and 5-HT1A receptors. *Br J Pharmacol*. 2016;173:815-825
28. England TJ, Hedstrom A, O'Sullivan SE, Woodhouse L, Jackson B, Sprigg N, et al. Remote Ischaemic Conditioning After Stroke Trial 2: a phase IIb randomised controlled trial in hyperacute stroke. *JAHA: Journal of the American Heart Association*. 2019;In Press
29. Missler U, Wiesmann M, Friedrich C, Kaps M. S-100 protein and neuron-specific enolase concentrations in blood as indicators of infarction volume and prognosis in acute ischemic stroke. *Stroke*. 1997;28:1956-1960
30. Zhao W, Meng R, Ma C, Hou B, Jiao L, Zhu F, et al. Safety and Efficacy of Remote Ischemic Preconditioning in Patients With Severe Carotid Artery Stenosis Before Carotid Artery Stenting: A Proof-of-Concept, Randomized Controlled Trial. *Circulation*. 2017;135:1325-1335
31. Meng R, Asmaro K, Meng L, Liu Y, Ma C, Xi C, et al. Upper limb ischemic preconditioning prevents recurrent stroke in intracranial arterial stenosis. *Neurology*. 2012;79:1853-1861
32. Meng R, Ding Y, Asmaro K, Brogan D, Meng L, Sui M, et al. Ischemic Conditioning Is Safe and Effective for Octo- and Nonagenarians in Stroke Prevention and Treatment. *Neurotherapeutics*. 2015;12:667-677
33. Zhao W, Zhang J, Sadowsky MG, Meng R, Ding Y, Ji X. Remote ischaemic conditioning for preventing and treating ischaemic stroke. *Cochrane Database Syst Rev*. 2018;7:Cd012503
34. Sloth AD, Schmidt MR, Munk K, Kharbanda RK, Redington AN, Schmidt M, et al. Improved long-term clinical outcomes in patients with ST-elevation myocardial infarction undergoing remote ischaemic conditioning as an adjunct to primary percutaneous coronary intervention. *Eur Heart J*. 2014;35:168-175
35. Hausenloy DJ, Candilio L, Evans R, Ariti C, Jenkins DP, Kolvekar S, et al. Remote Ischemic Preconditioning and Outcomes of Cardiac Surgery. *N Engl J Med*. 2015;373:1408-1417
36. Meybohm P, Bein B, Brosteanu O, Cremer J, Gruenewald M, Stoppe C, et al. A Multicenter Trial of Remote Ischemic Preconditioning for Heart Surgery. *N Engl J Med*. 2015;373:1397-1407
37. Kottenberg E, Musiolik J, Thielmann M, Jakob H, Peters J, Heusch G. Interference of propofol with signal transducer and activator of transcription 5 activation and cardioprotection by remote ischemic preconditioning during coronary artery bypass grafting. *J Thorac Cardiovasc Surg*. 2014;147:376-382
38. Botker HE, Kharbanda R, Schmidt MR, Bottcher M, Kaltoft AK, Terkelsen CJ, et al. Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. *Lancet*. 2010;375:727-734
39. White SK, Frohlich GM, Sado DM, Maestrini V, Fontana M, Treibel TA, et al. Remote ischemic conditioning reduces myocardial infarct size and edema in patients with ST-segment elevation myocardial infarction. *JACC Cardiovasc Interv*. 2015;8:178-188
40. Haller PM, Vargas KG, Haller MC, Piackova E, Wojta J, Gyongyosi M, et al. Remote ischaemic conditioning for myocardial infarction or elective PCI: systematic review and meta-analyses of randomised trials. *Eur Heart J Acute Cardiovasc Care*. 2018:2048872618784150
41. Gaspar A, Lourenco AP, Pereira MA, Azevedo P, Roncon-Albuquerque R, Jr., Marques J, et al. Randomized controlled trial of remote ischaemic conditioning in ST-elevation myocardial infarction as adjuvant to primary angioplasty (RIC-STEMI). *Basic Res Cardiol*. 2018;113:14
42. Vanezis AP, Arnold JR, Rodrigo G, Lai FY, Debiec R, Nazir S, et al. Daily remote ischaemic conditioning following acute myocardial infarction: a randomised controlled trial. *Heart*. 2018;104:1955-1962
43. Hausenloy DJ, Kharbanda RK, Moller UK, Ramlall M, Aaroe J, Butler R, et al. Effect of remote ischaemic conditioning on clinical outcomes in patients with acute myocardial infarction (CONDI-2/ERIC-PPCI): a single-blind randomised controlled trial. *Lancet*. 2019

44. Hauerslev M, Mork SR, Pryds K, Contractor H, Hansen J, Jespersen NR, et al. Influence of long-term treatment with glyceryl trinitrate on remote ischemic conditioning. *Am J Physiol Heart Circ Physiol*. 2018;315:H150-h158
45. Zhang SZ, Wang NF, Xu J, Gao Q, Lin GH, Bruce IC, et al. Kappa-opioid receptors mediate cardioprotection by remote preconditioning. *Anesthesiology*. 2006;105:550-556
46. Jensen RV, Stottrup NB, Kristiansen SB, Botker HE. Release of a humoral circulating cardioprotective factor by remote ischemic preconditioning is dependent on preserved neural pathways in diabetic patients. *Basic Res Cardiol*. 2012;107:285
47. Bath PM, Lees KR, Schellinger PD, Altman H, Bland M, Hogg C, et al. Statistical analysis of the primary outcome in acute stroke trials. *Stroke*. 2012;43:1171-1178
48. Zhang L, Diao Y, Chen G, Tanaka A, Eastwood GM, Bellomo R. Remote ischemic conditioning for kidney protection: A meta-analysis. *J Crit Care*. 2016;33:224-232
49. Rockwood K, Song X, MacKnight C, Bergman H, Hogan DB, McDowell I, et al. A global clinical measure of fitness and frailty in elderly people. *Cmaj*. 2005;173:489-495
50. von Kummer R, Broderick JP, Campbell BC, Demchuk A, Goyal M, Hill MD, et al. The Heidelberg Bleeding Classification: Classification of Bleeding Events After Ischemic Stroke and Reperfusion Therapy. *Stroke*. 2015;46:2981-2986
51. Anonymous. Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *European Heart Journal*. 2000;21:1502-1513
52. Krongold M, Almekhlafi MA, Demchuk AM, Coutts SB, Frayne R, Eilaghi A. Final infarct volume estimation on 1-week follow-up MR imaging is feasible and is dependent on recanalization status. *Neuroimage Clin*. 2015;7:1-6
53. England TJ, Abaei M, Auer DP, Lowe J, Russell N, Walker M, et al. Stem-cell trial of recovery enhancement after stroke 2 (STEMS2). Randomised placebo-controlled trial of granulocyte-colony stimulating factor in mobilising bone marrow stem cells in sub-acute stroke. *Stroke*. 2012;43:405-411
54. Battey TW, Karki M, Singhal AB, Wu O, Sadaghiani S, Campbell BC, et al. Brain edema predicts outcome after nonlacunar ischemic stroke. *Stroke*. 2014;45:3643-3648
55. Yoo RE, Yun TJ, Yoo DH, Cho YD, Kang HS, Yoon BW, et al. Monitoring cerebral blood flow change through use of arterial spin labelling in acute ischaemic stroke patients after intra-arterial thrombectomy. *Eur Radiol*. 2018;28:3276-3284
56. Gray LJ, Bath PM, Collier T. Should stroke trials adjust functional outcome for baseline prognostic factors? *Stroke*. 2009;40:888-894
57. Sprigg N, Flaherty K, Appleton JP, Al-Shahi Salman R, Bereczki D, Beridze M, et al. Tranexamic acid for hyperacute primary IntraCerebral Haemorrhage (TICH-2): an international randomised, placebo-controlled, phase 3 superiority trial. *Lancet*. 2018;391:2107-2115
58. Sena ES, Briscoe CL, Howells DW, Donnan GA, Sandercock PA, Macleod MR. Factors affecting the apparent efficacy and safety of tissue plasminogen activator in thrombotic occlusion models of stroke: systematic review and meta-analysis. *J Cereb Blood Flow Metab*. 2010;30:1905-1913
59. Ehrenreich H, Weissenborn K, Prange H, Schneider D, Weimar C, Wartenberg K, et al. Recombinant human erythropoietin in the treatment of acute ischemic stroke. *Stroke*. 2009;40:e647-656
60. Bath PM, Gray LJ, Collier T, Pocock S, Carpenter J. Can we improve the statistical analysis of stroke trials? Statistical reanalysis of functional outcomes in stroke trials. *Stroke*. 2007;38:1911-1915