This supplement contains:

1. Final SAP (March 20, 2023)
2. Summary of changes to SAP (February 9, 2018 to March 20 2023)
3. Original SAP (February 9, 2018)

Statistical Analysis Plan

TRIAL FULL TITLE	Remote ischemic conditioning in patients with acute stroke: a multicenter randomized, patient-assessor blinded, sham-controlled study.
SAP VERSION	1
SAP VERSION DATE	20.03.2023
TRIAL STATISTICIAN	Jan Brink Valentin
Protocol Version (SAP associated with)	*version 8.2 (31March2020)*
TRIAL PRINCIPAL INVESTIGATOR	Grethe Andersen
SAP AUTHOR(s)	Rolf A. Blauenfeldt

1 SAP Signatures

I give my approval for the attached SAP entitled RESIST dated 20.03.2023

Study coordinator & Investigator

Signature: REDACTED

Date: 21.03.2023

Statistician

Name:

Signature: REDACTED

Date: 22.03.2022

Principal Investigator

Name:

Signature: REDACTED

Date: 21 mar 2023

2 Table of Contents

1 SAP Signatures 1
2 Table of Contents 2
3 Study Objectives and Endpoints 3
3.1 Study Objectives 3
3.2 Endpoints 4
4 Study Methods 5
4.1 General Study Design and Plan 5
4.2 Inclusion-Exclusion Criteria and General Study Population 7
Inclusion criteria (prehospital) 7
Exclusion criteria 7
Final in-hospital inclusion criteria 7
4.3 Randomization and Blinding 8
4.4 Study Assessments 8
Table 1: Study procedures table 8
5 Sample Size 9
5.1 Timing of Analyses 11
5.2 Analysis Populations 11
5.2.1 Target population (Modified Intention to Treat) 11
5.2.2 Per Protocol Population 11
5.2.3 Safety Population (intention-to-treat) 11
5.2.4 Multi-center Studies 12
5.3 Missing Data 12
5.4 Interim Analyses and Data Monitoring 12
5.5 Source data access and monitoring (good clinical practice) 13
6 Summary of Study Data 14
6.1 Subject Disposition 14
6.2 Demographic and Baseline Variables 14
6.3 Coding system for MACCE and Recurrent ischemic events: 16
Recurrent ischemic vascular events at 3 and 12 months in AIS patients 16
6.4 Treatment protocol for study intervention 16
6.5 Treatment adherence 17
7 Efficacy Analyses 18
7.1 Secondary Efficacy Analyses 19
7.1.1 Secondary Analyses of Primary Efficacy Endpoint 21
8 Safety Analyses 21
9 References 22
10 Listing of Tables, Listings and Figures 23

3 Abbreviations

AE	Adverse event
AIS	Acute ischemic stroke
AR	Adverse reaction
CT	Computed tomography
CPSS	Cincinnati Prehospital Stroke Scale
DWI	Diffusion-weighted imaging
DMC	Data Monitoring Committee and Endpoints Validation Committee
ICH	Intracerebral hemorrhage
END	Early neurological deterioration
EVT	Endovascular treatment
LVO	Large Vessel Occlusion
miRNA	Micro ribonucleic acid
mPTP	Mitochondrial permeability transition pore
MRI	Magnetic resonance imaging
NPR	Danish National Patient Register
PreSS	Prehospital Stroke Score
PWI	Perfusion-weighted imaging
RIC	Remote ischemic Conditioning
RIPerC	Remote Ischemic perconditioning
RIPreC	Remote ischemic preconditioning
RIPostC	Remote ischemic postconditioning
IV tPA	Intravenous thrombolysis/recombinant tissue-type plasminogen activator
SAE	Serious adverse event
SAR	Serious adverse reaction
TIA	Transient ischemic attack
TIA (DWI+)	Transient ischemic attack with a DWI-positive lesion on MRI
TMG	Trial Management Group
TSC	Trial Steering Group

4 Study Objectives and Endpoints

4.1 Study Objectives

To determine whether Remote Ischemic Conditioning (RIC) improves the modified Rankin Scale score (mRS) at 3 months in patients with acute stroke, including acute ischemic stroke (AIS) and intracerebral hemorrhage (ICH)

Secondary aims include the association between RIC and secondary and exploratory endpoints listed below. In addition,

- Predictive abilities of Glial Fibrillary Acidic Protein (GFAP) and occludin in prehospital obtained blood samples combined with prehospital stroke severity to differentiate hemorrhagic from ischemic stroke and to grade ischemic stroke severity (*substudy at Aarhus University Hospital*)

- Diagnostic abilities of a prehospital microRNA and extracellular vesicles blood samples profile combined with prehospital stroke severity on the differentiation of hemorrhagic from ischemic stroke and to grade ischemic stroke severity (*substudy at Aarhus University Hospital*)
- microRNA and extracellular vesicle profile of RIC-induced neuroprotection at baseline (*substudy at Aarhus University Hospital*)
- Characterization of rheoerythrocrine dysfunction (RBC deformability, eryNOS3 and plasma nitrite) in RIC vs Sham-RIC treated stroke patients and its possible association to improved short term (24 hour) or long term (90 day mRS) clinical outcome or imaging biomarkers (DWI infarct growth) (*substudy at Aarhus University Hospital*)

4.2 Endpoints

Primary endpoint

- Clinical outcome (mRS) at 3 months in acute stroke (AIS and ICH)

Secondary endpoints

- Prehospital stroke score (PreSS) during 24 hours in all randomized patients
- Clinical outcome (mRS) at 3 months in subgroups: AIS, IV tPA/EVT treated AIS and ICH
- Prehospital stroke score (PreSS) during 24 hours in subgroups: IV tPA/EVT treated AIS and ICH.
- Complete remission of symptoms within 24 hours (TIA)
- Major Adverse Cardiac and Cerebral Events (MACCE) and recurrent ischemic events based on registry data at 3 and 12 months in ICH, AIS patients, TIA and non-vascular diagnosis
- Three-month and one-year mortality in AIS, ICH, and overall
- Early and very early neurological improvement in AIS and ICH patients
- Bed-day use at 3 and 12 months and quality of life measures at 3 months
- Clinical outcome (mRS) at 3 months in patients with AIS and ICH and extended remote ischemic postconditioning protocol (*substudy at Aarhus University Hospital*)
- Large vessel occlusion (LVO) eligible to EVT treatment in patients with AIS (*substudy at Aarhus University Hospital*)

Exploratory endpoint

- Hematoma reabsorption rate (7 days) in patients with ICH
- Acute (24-hour) hematoma expansion in patients with ICH
- Infarct growth in AIS patients and hematoma growth in ICH patients
- Modulation of coagulation (*substudy at Aarhus University Hospital*)
- Non stroke patients in all randomized patients

- One week blood-pressure reduction in AIS and ICH patients (*substudy at Aarhus University Hospital)*

5 Study Methods

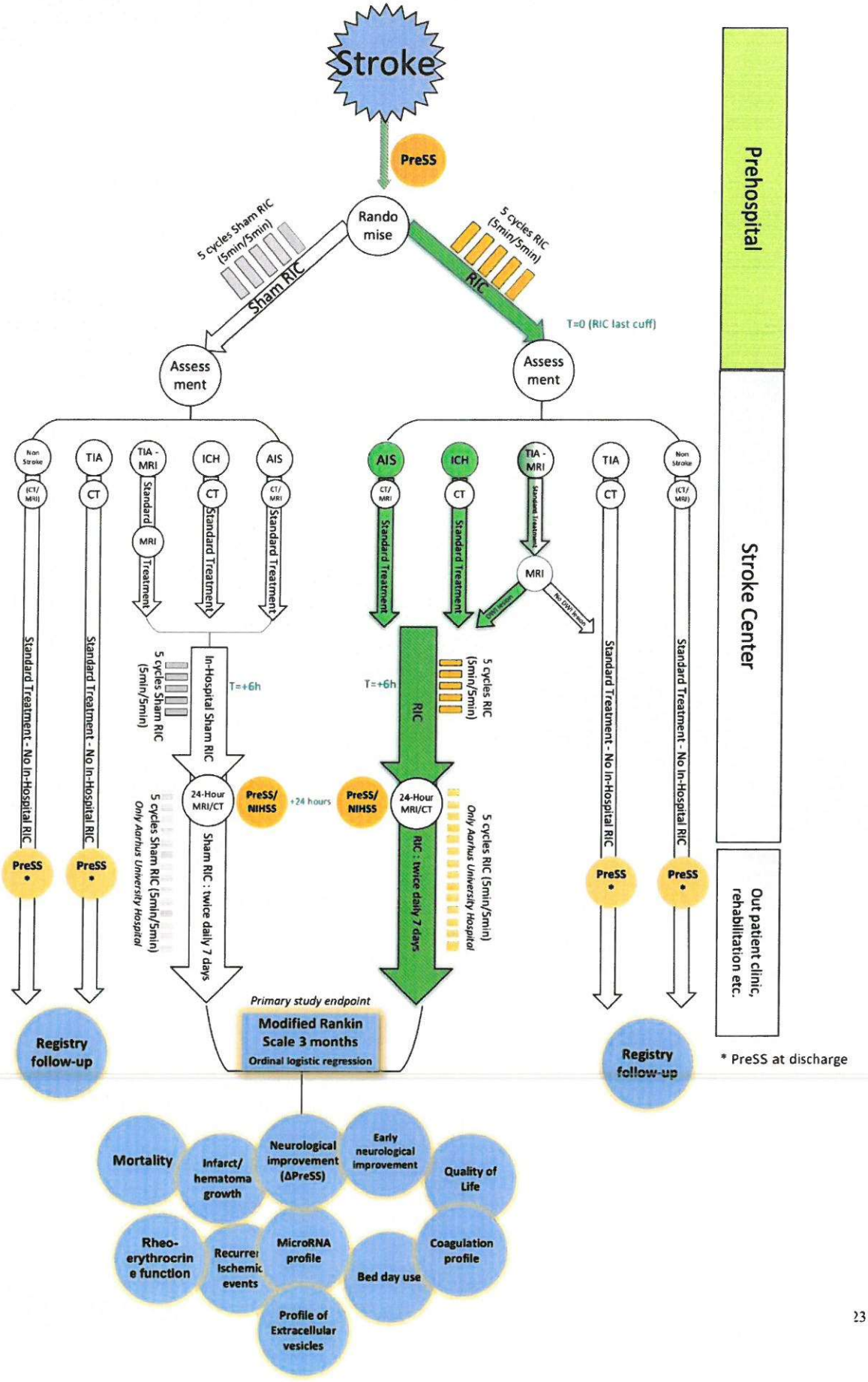
5.1 General Study Design and Plan

Study type: Multicenter, prospective, randomized, patient-assessor blinded, sham-controlled trial.

Type of Comparison: superiority

Type of control: Sham controlled
Level and method of blinding: Patient and assessor blinded.
Randomization: 1:1 with stratification on age, center and the Prehospital Stroke Score (PreSS).
Study procedures are visualized in Figure 1 and Table1

Figure 1: Study flowchart


Stroke
PreSS
Rando mise
5 cycles Sham RIC (5min/5min)
Sham RIC
5 cycles RIC (5min/5min)
RIC
T=0 (RIC last cuff)
Assess ment
Assess ment
Prehospital
Stroke Center
Out patient clinic, rehabilitation etc.
Non Stroke
TIA
TIA - MRI
ICH
AIS
(CT/ MRI)
CT
Standard MRI
Standard Treatment
Standard Treatment - No In-Hospital RIC
5 cycles Sham RIC (5min/5min)
In-Hospital Sham RIC
T=+6h
24-Hour MRI/CT
PreSS/ NIHSS
+24 hours
Sham RIC : twice daily 7 days
Only Aarhus University Hospital
5 cycles RIC (5min/5min)
RIC
RIC : twice daily 7 days
Primary study endpoint
Modified Rankin Scale 3 months
Ordinal logistic regression
Registry follow-up
* PreSS at discharge
Mortality
Infarct/ hematoma growth
Neurological improvement (ΔPreSS)
Early neurological improvement
Quality of Life
Rheo-erythrocrin e function
Recurrei Ischemic events
MicroRNA profile
Bed day use
Coagulation profile
Profile of Extracellular vesicles

5.2 Inclusion-Exclusion Criteria and General Study Population

All patients with a prehospital putative stroke who meet the study criteria will be included.

- Depending on the prehospital randomization (RIC versus sham), patients with AIS* and ICH will continue RIC or sham-RIC treatment for an extended period (after 6 hours at all centers and twice daily for 7 days at Aarhus University Hospital)

**Including patients with a transient ischemic attack and evident acute ischemic lesion on diffusion weighted imaging (DWI) – magnetic resonance imaging (MRI)*

Inclusion criteria (prehospital)

- Male and female patients (≥ 18 years)
- Prehospital putative stroke
- Onset of stroke symptoms < 4 hours before RIC
- Independent in daily living before symptom onset (mRS ≤ 2)

Exclusion criteria

- Intracranial aneurisms, AV malformation, cerebral neoplasm, abscess or progressive neurodegenereative disease
- Pregnancy*
- Severe peripheral arterial disease in the upper extremities
- Concomitant acute life-threatening medical or surgical condition
- AV shunt in the arm selected for RIC

**Women of child-bearing age should be asked about their use of safe birth control methods (contraceptive pill, intrauterine devices both hormonal and non-hormonal, hormonal implants, hormonal depot injection and transdermal hormonal patch). If pregnancy cannot be ruled out in the prehospital phase the patient can't be included. Women with a safe birth control method should be encouraged to use this method during the entire period of active RIC treatment.*

Final in-hospital inclusion criteria

- AIS (including DWI-positive TIA patients)

  or

- ICH

5.3 Randomization and Blinding

The patient will be randomized to standard treatment with RIC or sham-RIC by the on call neurologist/vascular neurologist at the receiving stroke center. The ambulance will contact the on call neurologist by telephone and describe the patient (standard operating procedure (SOP) in Denmark). The randomization is based on a secure web site providing computer-generated blocked randomization lists stratified by the center. The online randomization is stratified by age, stroke center and the Prehospital Stroke Score (PreSS) using a randomized block design. The PreSS score consists of the Cincinnati Prehospital Stroke Scale (CPSS) with an additional opportunity to report other neurological symptoms (e.g. ataxia, sensory disturbances and visual field loss), and PASS (Prehospital Acute Stroke Severity Scale)[1,2]. Prehospital personnel participating in RESIST are trained in identifying stroke symptoms included in PreSS[3]. The on call neurologist will make an assessment based on all available information whether the patient is eligible to participate in RESIST. Randomization is performed in the prehospital setting. Each on call neurologist participating in the study will receive unique access and will have no influence on the randomization process. All neurologists on call will be educated and trained in performing evaluation of potential eligible study candidates and to perform the online randomization during the telephone call with the ambulance personnel. There is always one neurologist on call in participating centers.

Treatment allocation

1:1 allocation

Blinding

Outcome assessment is blinded to the treatment arm and obtained by a clinical or telephone assessment of the level of dependency and need for help in daily activities (mRS) **(Primary study endpoint)**.

No information regarding randomization status will be recorded in the patient record.

Patients and the assessors of endpoints are blinded.

5.4 Study Assessments

Table 1: Study procedures table

	Acute prehospital and in-hospital phase					+ 6 hour	+ 24 hour			+ 7days		3 months	12 months
	Prehosp. stroke score (PreSS)[1]	Prehosp blood-samples[1]	RIC/Sham-RIC	In-hospital assessment and blood-samples[2]	CT/MRI baseline	+6 hour RIC/sham-RIC[4]	PreSS 24h[2]	CT/MRI 24h[3]	24-hour blood-samples[2]	Extended (7 days) RIC/sham-RIC[5]	CT-7 days[3]	mRS 3-months[7]	Clinical events during follow-up[6]
TIA (DWI-) -all	X	(X)*	X	X	X		X						X
Non-stroke - all	X	(X)*	X	X	X		X						X
AIS incl. TIA	X		X	X	X	X	X	X				X	X

(DWI+) – Other¥													
ICH - Other¥	X		X	X	X (CT)	X	X					X	X
AIS incl. TIA (DWI+) – AUH*	X	(X)	X	X	X(MRI)	X	X	X (MRI)	~~(X)~~	X		X	X
ICH – AUH*	X	(X)	X	X	X	X	X	X (CT)	~~(X)~~	X	X (CT)	X	X

**AUH = Aarhus University Hospital , ¥ Other= Other participating stroke centers*

[1] Prehospital stroke score (PreSS) and bloodsamples.

[2] Acute assessment at the Stroke Center. NIHSS, blood samples (21,5mL blood)-and ECG (standard). Assessment of neurological symptoms at 24-hour: NIHSS. Patients with non stroke diagnosis and TIA without a DWI-MRI lesion, who are discharged before 24 hours will be scored using PreSS at discharge. Patients, who had blood samples taken in the ambulance and upon stroke center arrival will have additional samples withdrawn at 2 (1-3) hours (5mL) after RIC/Sham-RIC completion and again 24(16-32) hours* after randomization (additional 21,5 mL blood) *RESIST blood samples only at Aarhus University Hospital.*

[3] Baseline CT is preferable for ICH patients (hospital SOP). ICH patients, at Aarhus University Hospital, will receive a 24 hour control CT (hospital SOP). If possible, a 24-hour MRI is performed in all included AIS patients with an MRI baseline scan. Furthermore, a 24-hour control CT will be provided for IV tPA-treated AIS patients, unless MRI can be performed and was the primary investigation. One week control CT will be performed, if possible, on ICH patients included at Aarhus University Hospital. End of study visit for non-vascular diagnosis and TIA (without DWI lesion) at stroke center discharge.

[4] RIC/sham-RIC at +6 hours from last RIC cuff

[5] RIC/sham-RIC twice daily for 7 days *(Only at Aarhus University Hospital)*

[6] Mortality, MACCE, and recurrent ischemic events are recorded using the Danish National Patient Register (LPR) and DSR at 6 and 15 months after the inclusion of the last patient.

[7] Assessments at 3 months by telephone or face-to-face (mRS and WHO-5). End-of-study (telephone or face-to-face) for AIS and ICH patients. WHO-5 will only be assessed by one assessor.

6 Sample Size

We estimate that a sample size of 1500 prehospital putative stroke patients will be required to achieve 1000 eligible AIS and ICH patients (primary study endpoint).

The treatment effect of RIC on long-term functional outcome is unknown. We have assumed a small but clinical significant neuroprotective effect corresponding to a 7% increased odds for a beneficial shift on the modified Rankin Scale. The sample size calculation is based on a simulation-based approach to the analysis of statistical power when ordinal logistic regression analysis is performed (significance level of 5%)

The statistical power was simulated at different hypothetical sample sizes (on the target population) (ranging from 200 to 1900) with 2000 simulation-runs performed at each step. Unpublished data on IV-tPA and/or EVT treated AIS patients and ICH patients from our institution were used:

3 months modified Rankin Scale distribution (proportions) in 2017 for patients with ICH or IV tPA/EVT treated AIS at Aarhus University Hospital.

Modfied Rankin Scale score	**mRS 0**	**mRS 1**	**mRS 2**	**mRS 3**	**mRS 4**	**mRS 5**	**mRS 6**
Proportion	0.139	0.273	0.141	0.110	0.145	0.07	0.126

Based on our previous trial experience[4] with prehospital remote ischemic conditioning we estimate that a sample of 1000 subjects with target diagnosis (AIS and ICH) will be feasible to include during the study period.

Including 1000 patients with target diagnosis provide sufficient power at an alfa-level (significance) of 5% to detect RIC treatment effects of the estimated 7 % *(see table below)*

Treatment effect (assumed cerrebroprotective)	5%	6%	**7%**
Sample (target diagnosis), *n*	1000	1000	**1000**
Power	66%	80%	**90%**
Alpha level (significance level)	5%	5%	**5%**

The estimated prehospital, randomized, sample:

Sample size, prehospital	**Proportion of randomized**	***n* =**
Target diagnosis (AIS and ICH), *n*	**67%**	**1000**
Non-vascular diagnosis	**27%**	**403**
TIA without DWI lesion	**4%**	**60**
Lost to follow-up	**2%**	**30**
Total		**1492**
Plan to include		**1500**

We therefore plan to include 1500 patients with a prehospital putative stroke in order to get 1000 patients with the target diagnosis of acute ischemic stroke and intracerebral hemorrhage. There is no planned replacement of patients lost to follow-up.

6.1 Timing of Analyses

- The final analysis will be performed after last three month visit for the last patients or if recommended by the DMSC due to safety reasons.
  AND
- Monitoring by regional good clinical practice (GCP) unit has completed
  AND
- Closure of database by signature of the PI
  AND
- Approval of the final SAP by the PI and trial statistician

6.2 Analysis Populations

6.2.1 Target population (Modified Intention to Treat)

- *All subjects who were prehospital randomized AND had an in hospital diagnosis of Acute ischemic stroke, Transient ischemic attack with an evident acute ischemic lesion on DWI-MRI or ICH patients.*
- *All subjects disregarding adherence to study intervention*

6.2.2 Per Protocol Population

- *All subjects in the "Target population" who adhere to the major criteria treatment adherence criteria in the protocol (e.g. all subjects who received at least 80% of assigned RIC/Sham cycles (each of 5min of cuff inflation and 5 minutes without inflation = 1 cycle)*
- *All subjects who did not substantially deviate from the protocol as to be determined on a per-subject basis at the trial steering committee immediately before database lock.*

6.2.3 Safety Population (intention-to-treat)

- *All subjects who were prehospital randomized, disregarding in-hospital diagnosis, but excluding subjects who refused to give written consent or withdrew their consent.*

Primary efficacy population: Target population (AIS, TIA +DWI-MRI lesion and ICH) (Modified Intention to Treat)

Predefined subgroup analysis of the primary endpoint:

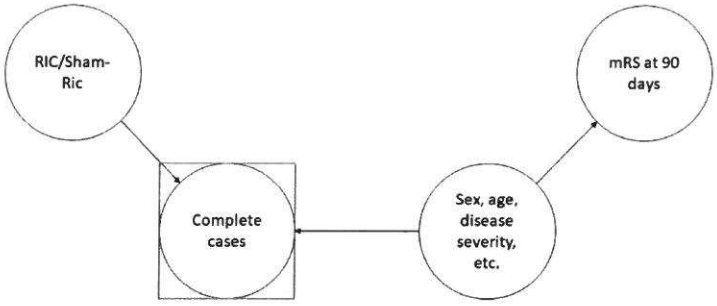
- AIS patients including TIA with DWI-MRI lesion
- AIS patients treated with intravenous thrombolysis (IVT)
- AIS patients treated with thrombectomy (EVT)
- AIS patients treated with reperfusion therapy (IVT and/or EVT)
- AIS patients not treated with reperfusion therapy (IVT and/or EVT)
- Intracerebral hemorrhage patients

6.2.4 Multi-center Studies

- Data from all centers will be analyzed as a whole
- Center effects will be presented in the subgroup analysis.

6.3 Missing Data

The association between treatment allocation and loss to follow-up is expected to be limited and explained by disease severity, pre-stroke functional level, sex, age, site, etc. The underlying assumption is depicted in the figure below. Thus, missing data incl. outcome is assumed to be missing at random and will be handled using multiple imputation chained equations with appropriate variables as model input.



In a complete case analysis selection bias may occur on account of underlying parameters being associated with dropout rate and outcome. We assume that these parameters are available in the data set.

The extent of missing data will be presented for outcome variables to quantify the extent of missing data.

6.4 Interim Analyses and Data Monitoring

We will perform an interim analysis for evaluation of the actual event rate after inclusion of 50% of the patients. If this shows a lower good outcome rate than expected, a new sample size calculation will be performed. Furthermore, the Data monitoring committee (DMC) will perform an independent safety analysis and will review the overall safety and efficacy according to the DMC charter (planned in the early phase of the trial, at 12 month and hereafter at least every 12. Months) An additional safety interim analysis of mortality and hematoma expansion in ICH patients will be performed. Interim analyses will be assessed using a level of significance of 1%.
The trial can be stopped at interim analyses for both futility, efficacy and safety reasons. Detailed description is found in the DMC charter.

Trial decision making and stopping rules

A Data Monitoring Committee (DMC) will be appointed before trial start and include a signed DMC charter. The DMC will review the accumulated data during RESIST trial and provide advice on the conduct of the trial to the Steering Committee.

The DMC should inform the Steering Committee
if, in their view:

- the results are likely to provide convincing evidence that one trial arm is clearly indicated or contraindicated, and there is a reasonable expectation that this new evidence would materially influence patient management

or

- it is beyond doubt that no clear outcome would be obtained (futility)

It is not expected that RESIST trial will be stopped for reasons of efficacy or futility based on observed treatment differences. A predefined pooling of individual patient data (Remote Ischemic conditioning in Stroke Collaboration, RISC) is planned. To dispel scepticism, a robust demonstration of efficacy of this simple intervention is required. The primary reason to recommend stopping the trial will be for safety reasons.

For Acute Ischemic Stroke and Intracerebral Hemorrhage:
For the primary endpoint the DMC will consider stopping if:

- There is a robust improvement in functional outcome after 3 months (modified Rankin Scale) for Acute Ischemic Stroke and Intracerebral haemorrhage in the sham (control) group compared to the intervention (RIC) group, achieving $p<0.01$.
- A substantial number of patients experience serious adverse events ($p<0.01$)

- If there is an increase in mortality or stroke recurrence for Acute Ischemic Stroke and Intracerebral Hemorrhage patients in the intervention (RIC) group compared to the control (sham) group, achieving $p<0.01$.

- If there is a clear evidence of hematoma growth among patients with ICH during the first 24-hour in the intervention group, achieving $p<0.01$

The DMC will make recommendations, which could include:

- No action needed, trial continues as planned
- Early stopping due, for example, to clear benefit or harm of a treatment, futility, or external evidence
- Stopping recruitment within a subgroup
- Extending recruitment (based on actual control arm response rates being different to predicted other than on emerging differences) or extending follow-up
- Approving and/or proposing protocol changes

6.5 Source data access and monitoring (good clinical practice)

Source data access and monitoring (good clinical practice)

Trial-related audits and/or monitoring will be provided by direct access to source data/documents. Local monitors from the Unit for Good Clinical Practice, Aarhus University, will perform the audit and monitoring. Audit and monitoring will, likewise, be perfomed by qualified local GCP-monitors when enrolment at other stroke centers in Denmark is starting. Before enrolment can start a trial monitoring plan must be available. The audit and monitoring process will involve a 100% monitoring of signed consent forms ("samtykkeerklæringer og fuldmagtserklæringer") and serious adverse events.

7 Summary of Study Data

- Summary tables will be structured (columns for each treatment and overall in the order: all subjects, Sham, RIC)
- Descriptive or summary statistics that will be displayed for continuous data and for categorical data.
- The analysis populations upon which the tables and figures will be based.
    - Summary statistics on target group (Table 1) and Safety population (all randomized) in supplemental materials.

All continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, median, 25% and 75% interquartile range. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. All summary tables will be structured with a column for each treatment in the order (Sham, RIC) and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

7.1 Subject Disposition

An overview of the time-dependent rates of recruitment will be provided.
Details on withdrawn consents and any reasons for that will be presented.
The study flowchart will adhere to CONSORT principles and a CONSORT statement will be made.
The reporting requirements of the regulatory agencies in Denmark will be met.

7.2 Demographic and Baseline Variables

All randomized (safety population): Demographic or baseline variables that will be included as a total and in each group (RIC and sham) are listed below and are intended for a supplemental table.

- Age, median (continuous)
- Female, (categorical)
- Prehospital Stroke Score (PreSS)
    - Prehospital Stroke Score item : Facial palsy (categorical, yes/no)
    - Prehospital Stroke Score item : Arm drift (categorical, yes/no)
    - Prehospital Stroke Score item : Incorrect months or age (categorical, yes/no)
    - Prehospital Stroke Score item : Speech difficulty (categorical, yes/no)
    - Prehospital Stroke Score item : eye deviation, eye muscle palsy (categorical, yes/no)
    - Prehospital Stroke Score item : Other (categorical, yes/no)

- Diagnosis (AIS, TIA +DWI-MRI lesion, ICH, TIA -DWI-MRI lesion and stroke mimics by category)

Target population: Demographic or baseline variables that will be included as a total and in each group (RIC and sham) are listed below. The anticipated statistical summary tables for the target population will include:

- Age, median (continuous)
- Female, (categorical, yes/no)
- Hypertension, (categorical, yes/no)
- Diabetes, (categorical, yes/no)
- Atrial fibrillation, (categorical, yes/no)
- Prior AIS, (categorical, yes/no)
- Prior TIA, (categorical, yes/no)
- Prehospital systolic blood pressure, (continuous)
- Prehospital diastolic blood pressure, (continuous)
- PreSS, median (continuous)
- Admission NIHSS, median (continuous)
- Discharge diagnosis (categorical, yes/no)
    - AIS
    - TIA
    - ICH
- Patients with acute ischemic stroke (categorical, yes/no)
- Stroke etiology (categorical, yes/no)
    - Large artery disease
    - Small vessel disease
    - Cardioembolic
    - Other/rare/unknown
- NIHSS, (continuous)
- Intravenous thrombolysis (categorical, yes/no)
- Admission to thrombolysis, minutes (continuous)
- Thrombectomy (categorical, yes/no)
- Door to groin puncture, minutes (continuous)
- Site of occlusion during angiography (categorical, yes/no)
    - Intracranial internal carotid artery
    - Main stem of middle cerebral artery
    - Branch from middle cerebral artery
    - Basilar artery
    - Other
    - No occlusion at time of EVT
- TICI 2b-3 reperfusion (categorical, yes/no)
- Patients with intracerebral hemorrhage (categorical, yes/no)
- Acute Glasgow coma scale score, (continuous)
- Deep hematoma localization (categorical, yes/no)
- Platelet inhibitor use (categorical, yes/no)
- Oral anticoagulation use (categorical, yes/no)

- Hematoma evacuation or external ventricular drainage (categorical, yes/no)

7.3 Coding system for MACCE and Recurrent ischemic events:

MACCE is defined as:

Cardiovascular events (cardiovascular death, myocardial infarction, acute ischemic or hemorrhagic stroke)
Cardiovascular death: Death from known cardiovascular cause or sudden death from unknown cause (no identified cause of death in medical history and/or autopsy)
Acute myocardial infarction: Admission with a discharge diagnosis of ST-elevation myocardial infarction (STEMI) and non-ST elevation myocardial infarction (NSTEMI) and unstable angina pectoris (UAP)

Stroke: Admission with a discharge diagnosis of acute ischemic or hemorrhagic stroke. Evaluation is performed using the Danish National Patient Register (LPR) and the DSR at two time points (6 and 15 months after the inclusion of the last patient).

Diagnosis of AIS/TIA, ICH and MI (STEMI, NSTEMI, and UAP) are made according to national clinical practice guidelines.
(http://neuro.dk/wordpress/nnbv/om-iskaemisk-apopleksi/ and http://nbv.cardio.dk/aks)

Recurrent ischemic vascular events at 3 and 12 months in AIS patients
Recurrent ischemic vascular events defined as:

AIS
TIA
MI, STEMI, and NSTEMI

See "CEC charter version 1.0 (30.08.2022)" and "event adjudication, definitions and code list version 1.2 (24.11.2022)"

7.4 Treatment protocol for study intervention

Remote Ischemic Conditioning device (RIC device)
The device is programmed to five cycles (50 minutes), each consisting of five minutes of cuff inflation followed by five minutes with a deflated cuff. The cuff pressure will be 200mmHg; but if initial systolic blood pressure is above 175 mmHg, the cuff is automatically inflated to 35 mmHg above the systolic blood pressure. This is done to account for a maximum cuff air leakage of 6mmHg per minute and maximum deviation in blood pressure measurement of 10mmHg. The maximum cuff pressure is 285 mmHg.
Sham – remote ischemic conditioning device (Sham-RIC device)
The device is programmed to five cycles (50 minutes), each consisting of five minutes of cuff inflation followed by five minutes with a deflated cuff. The cuff pressure during inflation will be 20mmHg.
Timestamps, blood pressure before RIC/sham-RIC and total RIC/sham-RIC cycles are recorded and stored on the device (accessible by *universal serial bus,* USB). The RIC and sham-RIC is pre-

programmed before being delivered to collaborating centers/ambulances. No data on conditioning cuff pressure will be displayed on device screen.
In cases of short transport time, the RIC/sham-RIC protocol continues during the initial assessment at the stroke center. RIC/sham-RIC stimulation is discontinued just prior to the MRI and continues afterwards if the protocol was insufficient (< 4 cycles). In the cases of insufficient treatment before MRI a complete RIC/sham-RIC protocol will be performed (5 cycles) as soon as possible after MRI. This is done to ensure that all participants receive at least 4 consecutive cycles of RIC/sham-RIC. In centers using CT, the RIC/sham-RIC protocol is continued (if possible) during the scan until a total of 5 cycles are reached. The device automatically stops treatment when the 5 cycles are completed. Except for RIC/sham-RIC, the prehospital observation and treatment are according to standard procedures in the ambulance.
***The use of** Stroke RIC/Sham-RIC **was** approved by the Danish Medicines Agency*

Initial remote ischemic conditioning
(all patients)
RIC/sham-RIC cuff will be placed on the non-paretic upper arm. The cuff is placed on the upper extremity on the same side of the suspected side of cerebral stroke, e.g. in right hemiparesis the cuff on the left arm, and in monosymptomatic aphasia the cuff is placed on the left arm.
Remote ischemic conditioning at +6 hours
(All patients with AIS, ICH – all centers)
At 6 hours after the first RIC/sham-RIC protocol (6 hours from the last cuff-off, respectively), another series of RIC/sham-RIC is performed; five cycles each consisting of 5 minutes of ischemia are followed by 5 minutes of reperfusion. *In the case of mechanical thrombectomy the +6 hours treatment may be delayed and will be started as soon as possible.*
Remote ischemic postconditioning (twice daily for 7 days)
(AIS and ICH – only at Aarhus university Hospital)
The post-conditioning stimulus will be applied twice daily in the first week. This occurs at pre-specified times: 08.00 PM and AM. However, RIC/sham-RIC can be applied no sooner than 10 hours and not later than 21 hours after the prehospital conditioning stimulus (last cuff).
Aarhus University Hospital: RIC/sham-RIC treatment will continue for a total of 7 days. If patients are discharged to their home or to another department, they will continue treatment according to written instructions, including application of the device for 7 days. After RIC/sham-RIC on day 7, the device is then returned to the Stroke Center.

7.5 Treatment adherence

Assessment of treatment adherence include:

Defining acceptable treatment adherence, the following definition was used:
At least 80% of the overall assigned RIC/sham cycles that were received was required for acceptable adherence to treatment.
For the practical application of the intervention 80% was accepted in each treatment period. Values below this level prompted questions on reasons for the reduced adherence to treatment.

Acute RIC		**+6 hours**		**+7days RIC** (Only Aarhus University Hospital)	
Planned	**Accepted**	**Planned**	**Accepted**	**Planned**	**Accepted**

5 cycles (5/5min) 100%	4 Cycles (5/5min) 80%	5 cycles (5/5min) 100%	4 Cycles (5/5min) 80%	100%	80%

Treatment adherence data:
Each device counts the number of complete cycles (5min cuff inflation and 5 minutes of cuff deflation) completed. Each cycle starts with cuff inflation. If the device is stopped prematurely (eg. After 5 minutes of cuff inflation) the current cycle will not be recorded.
If a device is not returned back to the stroke center: Adherence is 0%

Adherence is calculated as:

$$Adherence\ (\%) = \frac{\text{Actual cycles (no.)}}{\text{Planned cycles (no.)}} x\ 100\%$$

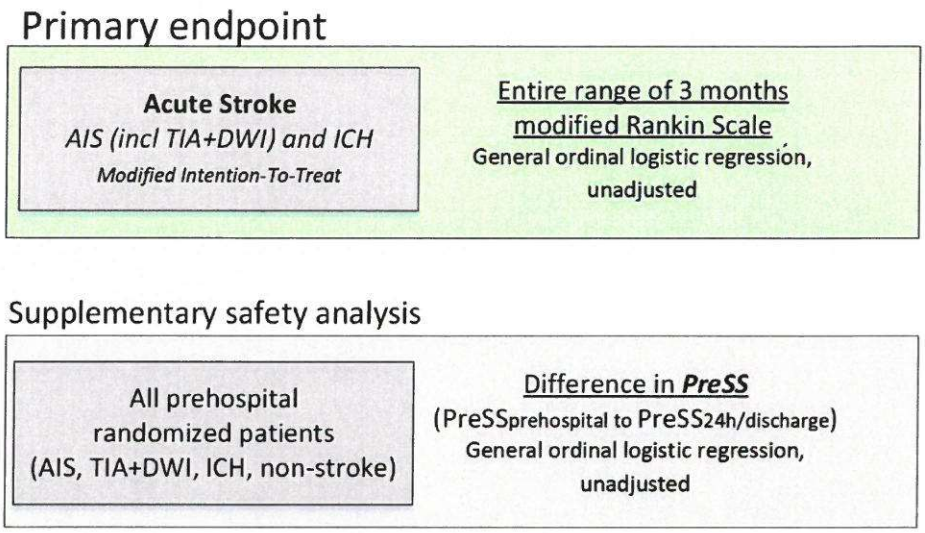
8 Efficacy Analyses

Primary study endpoint:

Odds ratio for shift in clinical outcome (mRS) at 3 months in acute stroke ***(Ordinal logistic regression analysis. Significance level of 5%)***

The **main analysis of the primary study endpoint** will be performed using the entire range of the modified Rankin Scale **(Ordinal logistic regression with a random effect on randomization blocks, otherwise unadjusted)** on the population fulfilling the ***in-hospital inclusion criteria (target population)***. The trial success analysis is performed on the **target population** which consists of prehospital randomized patients with an in-hospital diagnosis of acute ischemic stroke (including TIA with a DWI lesion) and intracerebral hemorrhage (ICH) regardless of adherence to investigational treatment (**modified Intention-To-Treat analysis**). *Treatment groups will be tested at the 2-sided 5% significance level.*

Supplementary safety analysis:
All patients randomized in RESIST will before randomization have a focal neurological deficit (documented on the prehospital stroke score). The presence or absence of specific focal neurological symptoms are assessed again after 24 hours using the same score as pre-randomization (PreSS) in all randomized patients (PreSS 24 hour/ at discharge). The primary safety analysis will be made on the **entire randomized population using the odds ratio for shift in prehospital stroke score** (PreSS) to document change in neurological deficits between pre-randomization and 24 hour/discharge **(Ordinal logistic regression with a random effect on randomization blocks, otherwise unadjusted)**. There has been no safety concern over RIC treatment in patients with non-vascular diagnosis[4,5,6,7,8]



If remote ischemic preconditioning is associated with ultra-early treatment effect and reduced symptom severity (in the ambulances), this could affect the in-hospital indication for IV-tPA and/or EVT (in the case of rapid improvement of symptoms or mild symptoms)
Because of this, a **supplementary analyses** using **ordinal logistic regression, adjusted for a possible** imbalances in reperfusion treatments (IV-tPA and EVT) between treatment arms will be made.

All analyses using ordinal logistic regression are supplemented a stacked bar chart (Grotta bar).

8.1 Secondary Efficacy Analyses

- Odds ratio for shift in prehospital stroke score (PreSS) (Supplementary safety endpoint, see above) (All randomized patients, ordinal logistic regression, significans level 5%)
- Odds ratio for shift in prehospital stroke score (PreSS) during the first 24 hours: (ordinal logistic regression analysis in the stroke subtypes, Significance level of 5%)
- Relative and absolute difference in proportion of patients with complete remission of symptoms within 24 hours (TIA; both with and without DWI) (All randomized patients, Binomial regression analysis with identity- and log-link function. Significance level of 5%)
- Odds ratio for shift in early neurological improvement in AIS and ICH *(ordinal logistic regression analysis. Significance level of 5%)*

  -Very early neurological improvement is defined as:
  Reduction in prehospital stroke score (PreSS) ≥ 1 points or resolution of symptoms at admission: Items on PreSS related to NIHSS baseline ΔpreSS = $PreSS_{prehospital}$ – same items on $NIHSS_{baseline}$

  -Early neurological improvement is defined as:

Reduction in NIHSS ≥ 4 (baseline versus 24-Hour NIHSS): $\Delta NIHSS = NIHSS_{baseline} - NIHSS_{24}$

-Reduction in prehospital stroke score (PreSS) ≥ 1 points or resolution of symptoms after 24 hours in subgroups: IV tPA/EVT treated AIS and ICH.
Reduction in NIHSS ≥ 4 (baseline versus 24-Hour NIHSS): $\Delta NIHSS = NIHSS_{baseline} - NIHSS_{24}$

-Improvement in median percent change in 24-hour NIHSS:
Median percentage change in NIHSS = $[(NIHSS_{baseline} - NIHSS_{24}) / NIHSS_{baseline}] \times 100$

- Odds ratio for shift in prehospital stroke score (PreSS) after 24 hours in subgroups: IV tPA/EVT treated AIS and ICH (ordinal logistic regression analysis, significance level of 5%).
- Relative and absolute difference in acute hematoma expansion in patients with ICH (Binomial regression analysis with identity- and log-link function, significance level of 5%)

  - Acute hematoma expansion in patients with ICH
  Hematoma expansion is defined as the difference in volume between the baseline and the 24-hour CT hematoma volume. Significant hematoma growth is defined as an absolute growth exceeding 6 mL or a relative growth of more than 33% from the initial CT[9]. (Binomial regression analysis with identity- and log-link function, significance level of 5%)

  -Difference in 7 days hematoma reabsorption rate in patients with ICH
  Hematoma reabsorption rate is defined as the difference in hematoma volume between the baseline and the 7-day CT hematoma volume. (Linear regression analysis with robust variance estimation, significance level of 5%)

- Difference in acute infarct growth in patients with AIS treated with IV-tPA and/or EVT (Linear regression analysis with robust variance estimation, significance level of 5%)

  -Acute infarct growth is defined as the difference in infarct volume between baseline and 24 hours on DWI-MRI. All patients with IV-tPA and/or EVT treated ischemic stroke and a baseline MRI will have an additional 24-hour MRI performed.

- Hazard rate ratio (HRR) of major adverse cardiac and cerebral events (MACCE) and recurrent ischemic events at 3 and 12 months in AIS patients *(Cox regression analysis, significance level of 5%)*

  -Definition see 6.3 Coding system for MACCE and Recurrent ischemic events:

- Relative and absolute three-month and one-year mortality (Binomial regression analysis with identity- and log-link function, significance level of 5%)

  -Definition see 6.3 Coding system for MACCE and Recurrent ischemic events:

- EVT-eligibility (MRI assessed) in RIC treated AIS patients with large vessel occlusion (LVO)
  - Relative and absolute difference in proportion of RIC treated AIS patients with LVO eligible to EVT treatment compared to standard treatment, adjusted for prehospital stroke severity (PreSS) and symptom duration (Binomial regression analysis with identity- and log-link function, significance level of 5%).

-Difference in MRI-DWI lesion volume in RIC/sham-RIC treated LVO AIS patients eligible to EVT treatment (Linear regression analysis with robust variance estimation, significance level of 5%).

8.1.1 Secondary Analyses of Primary Efficacy Endpoint

- A subgroup analysis will be performed on:
- Acute ischemic stroke patients including TIA with DWI-MRI lesion
- Acute ischemic stroke patients treated with intravenous thrombolysis (IVT)
- Acute ischemic stroke patients treated with thrombectomy (EVT)
- Acute ischemic stroke patients treated with IVT and/or EVT
- AIS patients not treated with reperfusion therapy (IVT and/or EVT)
- Intracerebral hemorrhage patients

AND the following characteristics will be included in subgroup analysis and imputation model:

- Age <65 and >=65years (categorical, yes/no)
- NIHSS ≤5 and NIHSS >5 (categorical, yes/no)
- Time from stroke onset to randomization (≤ 60 minutes vs >60 minutes)
- Hypertension (categorical, yes/no)
- Female (categorical, yes/no)
- Hypertension (categorical, yes/no)
- Diabetes (categorical, yes/no)
- Atrial fibrillation (categorical, yes/no)
- Discharge diagnosis
    - AIS (categorical, yes/no)
    - TIA (categorical, yes/no)
    - ICH (categorical, yes/no)
- Stroke etiology (categorical)
    - Large artery disease (categorical, yes/no)
    - Small vessel disease (categorical, yes/no)
    - Cardioembolic (categorical, yes/no)
    - Other/rare/unknown (categorical, yes/no)
- Center (AUH, OUH, Gødstrup, AaUH) (categorical, 4 categories)
- Adherence (<80 vs >=80 years) (categorical, yes/no)
- Door to groin puncture, minutes (continuous)
- Deep hematoma localization (categorical, yes/no)

Results will be presented in a forest plot with 95% confidence intervals (CI).

9 Safety Analyses

All adverse events including death will be summarized tables for the target population and the safety population group.

Tables providing an overview on:

<u>One Table with events during the study:</u>

- Serious Adverse Events: A table of all serious adverse events SAE and SADE (Serious Adverse Device Effect) or ASADE (Unanticipated Serious Adverse Device Effect (USADE), (See Adverse Events definition below) and whether it was related (related, probable, possible) or unrelated (unlikely, not related, undetermined, not relevant)
- Adverse Events (all events not included above) and whether it was related (related, probable, possible) or unrelated (unlikely, not related, undetermined, not relevant)
- Tables will grouped by type of event and/or organ system, with number and frequency of such events in each arm/group of the clinical study

One Table with death and cardiovascular events during the study:

Mortality and vascular events (MACCE) and recurrent ischemic events as classified by CEC

- Mortality (all cause) and cardiovascular and non-cardiovascular mortality within the two groups of the clinical study, grouped by event type, with number and frequency of such events in each arm/group of the clinical study.
- Vascular events (MACCE) and recurrent ischemic events Other (Not Including Serious) within the two groups of the clinical study, grouped by event type, with number and frequency of such events in each arm/group of the clinical study.
- Its relation to study intervention will be presented "related" (related, probable, possible) or "unrelated" (unlikely, not related, undetermined, not relevant)

10 References

1. Hastrup S, Damgaard D, Johnsen SP, Andersen G. Prehospital Acute Stroke Severity Scale to Predict Large Artery Occlusion: Design and Comparison With Other Scales. *Stroke*. 2016;47(7):1772-1776. doi:10.1161/STROKEAHA.115.012482
2. Kothari RU, Pancioli A, Liu T, Brott T, Broderick J. Cincinnati Prehospital Stroke Scale: reproducibility and validity. *Ann Emerg Med*. 1999;33(4):373-378.
3. Gude MF, Blauenfeldt RA, Behrndtz AB, et al. The Prehospital Stroke Score and telephone conference: A prospective validation. *Acta Neurol Scand*. 2022;145(5):541-550. doi:10.1111/ANE.13580
4. Hougaard KD, Hjort N, Zeidler D, et al. Remote ischemic perconditioning as an adjunct therapy to thrombolysis in patients with acute ischemic stroke: A randomized trial. *Stroke*. 2014;45(1):159-167. doi:10.1161/STROKEAHA.113.001346
5. Le Page S, Bejan-Angoulvant T, Angoulvant D, Prunier F. Remote ischemic conditioning and cardioprotection: a systematic review and meta-analysis of randomized clinical trials. *Basic Res Cardiol*. 2015;110(2):11. doi:10.1007/s00395-015-0467-8
6. Bøtker HE, Kharbanda R, Schmidt MR, 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(9716):727-734. doi:10.1016/S0140-6736(09)62001-8
7. Hausenloy DJ, Candilio L, Evans R, et al. Remote Ischemic Preconditioning and Outcomes of Cardiac Surgery. *N Engl J Med*. 2015;373(15):1408-1417. doi:10.1056/NEJMoa1413534
8. Laiwalla AN, Ooi YC, Liou R, Gonzalez NR. Matched Cohort Analysis of the Effects of Limb Remote Ischemic Conditioning in Patients with Aneurysmal Subarachnoid Hemorrhage. *Transl Stroke Res*. 2016;7(1):42-48. doi:10.1007/s12975-015-0437-3
9. Demchuk AM, Dowlatshahi D, Rodriguez-Luna D, et al. Prediction of haematoma growth and outcome in patients with intracerebral haemorrhage using the CT-angiography spot sign (PREDICT): a prospective observational study. *Lancet Neurol*. 2012;11(4):307-314.

doi:10.1016/S1474-4422(12)70038-8

11 Listing of Tables, Listings and Figures

Disposition / study flowchart is planned for table of Figure 1.xxx
Primary and secondary efficacy and safety endpoints – Table2 or Figure 2 (horizontal stacked bar chart – "grotta bars")
Safety is planned as Table or Figure 3
Subgroup analysis is planned as Table 4 or Figure 4

The following supplemental tables/figures are planned
Primary Endpoint Analysis without Imputation of Missing Data
Primary Endpoint – Sensitivity Analyses
Safety Analysis – Serious Adverse Events

Supplementary Tables
Table S1: Baseline characteristics of Intention to Treat Population
Table S2: Primary and Secondary Efficacy Outcomes – Per-Protocol Population
Table S3: Additional Efficacy and Safety Outcomes – modified Intention to Treat Population
Table S4: Device treatment adherence
Table S5: Death and Serious Adverse Events
Table S6: Category of diagnosis in the stroke mimics group
Supplementary Figures
Figure S1. Additional Subgroup Analysis.
Figure S2. Subgroup Analysis – Pre-Specified Subgroups (Forestplot)
Figure S3. Investigational device photo.

RESIST - database lock

TRIAL FULL TITLE	Remote ischemic conditioning in patients with acute stroke: a multicenter randomized, patient-assessor blinded, sham-controlled study.
TRIAL STATISTICIAN	Jan Brink Valentin
Protocol Version	*version 8.2 (31March2020)*
SAP version	*Version 1 (27March2023)*
TRIAL PRINCIPAL INVESTIGATOR	Grethe Andersen
AUTHOR(s)	Rolf A. Blauenfeldt
Version	*v1.0 21March2023*

1 Database lock Signatures

I give my approval for the lock of the RESIST database.

Study coordinator & Investigator

Name: Rolf A. Blauenfeldt

Signature: *REDACTED*

Date: March 28, 2023

Principal Investigator

Name: Grethe Andersen

Signature: *REDACTED*

Date: 28. marts 2023

Trial Statistician

Name: Jan Brink Valentin

Signature: *REDACTED*

Date: 2023-03-27

2. Summary of changes to the statistical analysis plan

From Nov 29, 2018 to March 20, 2023

Changes	Date of change	Document
Change of primary endpoint: From difference in Prehospital Stroke Scale (PreSS) score during 24 hour **to difference in 90-day functional outcome (modified Rankin Scale)**	**Nov 29, 2018**	Please see summary of Protocol changes
Secondary endpoint of 7 day hematoma reabsorption rate in ICH was added	**Nov 29, 2018**	Please see summary of Protocol changes
Planned demographics and patient characteristics to include in Table 1	**March 20, 2023**	SAP page 14-15
Detailed description on baseline demographics and patient characteristics to include in the planned subgroup analysis based	**March 20, 2023**	SAP page 21
Description on how adverse events will be presented	**March 20, 2023**	SAP page 21 and 22
Change in planned imputation method for missing data: Change from predictive mean matching to chained equations	**March 20, 2023**	SAP page 12
Overview of planned tables and figures	**March 20, 2023**	SAP page 23

3. Original statistical analysis plan (SAP)

The original statistical analysis plan was included in the protocol (clinical investigation plan February 9, 2018).

The following section contains these elements from the protocol

Statistics

Primary study endpoint:

- Difference in prehospital stroke score (PreSS) during the first 24 hours:

  ΔPreSS = $PreSS_{prehospital}$ – $PreSS_{24\text{-}hours}$ ***(Ordinal logistic regression analysis in the per-protocol population. Significance level of 5%)***

  The **main analysis of the primary study endpoint** will be performed using the entire range of the difference in PreSS score **(ordinal logistic regression, unadjusted)** on the ***per-protocol population.*** The per-protocol cohort consists of prehospital randomized patients with an in-hospital diagnosis of acute ischemic stroke with complete investigational treatment and endpoint assessment as defined in the clinical investigational plan.

  A supplementary analysis will be made on the **Intention-to-treat (ITT) population**. The ITT cohort includes all randomized subjects. In non-stroke diagnosis and TIA patients without MRI-DWI lesion, who are discharged before 24-hours, the stroke severity will be documented as *PreSS at discharge (performed by the on call neurologist)*
  Furthermore, a supplementary analyses using multivariable regression will be done in order to account for any imbalances in prognostic factors including the distribution of patients receiving reperfusion therapy between the two treatment arms

- Difference in acute hematoma expansion in patients with ICH (safety outcome) (Binomial regression analysis. Intention-to-treat analysis (all randomized patients with an intracerebral hemorrhage, ICH) Significance level of 5%)

Secondary study endpoints (selected):

- Difference in clinical outcome (mRS) at 3 months in acute ischemic stroke *(ordinal logistic regression analysis. Significance level of 5%)*
- Difference in Early neurological improvement in AIS and ICH *(ordinal logistic regression analysis. Significance level of 5%)*
- Difference in prehospital stroke score (PreSS) after 24 hours in subgroups: IV tPA/EVT treated AIS and ICH. (ordinal logistic regression analysis, significance level of 5%)
- Major adverse cardiac and cerebral events (MACCE) and recurrent ischemic events at 3 and 12 months in AIS patients *(Cox regression analysis, significance level of 5%)*

- Three-month and one-year mortality *(Two sample test of proportion (Chi-square test), significance level of 5%)*
- EVT-eligibility (MRI assessed) in RIC treated AIS patients with large vessel occlusion (LVO) *(Binomial regression analysis. Significance level of 5%)*

Sample Size

The sample size calculation is based on data from IV tPA-treated AIS patients from our institution using an estimated baseline and 24-hour PreSS (from NIHSS baseline and 24 hour). The proportion of AIS patients with a significant reduction of neurological impairment during 24 hours (PreSS score reduction of ≥ 1 point) was 0.43. To detect a 7% absolute increase in neurological improvement in the RIC-treated AIS group at a power of 80% and a significance level of 5%, a sample size of at least 1,380 will be required. To account for non-stroke diagnosis (estimated 24%), ICH (estimated 8%), TIA (estimated 8%), withdrawal and loss to follow-up (estimated 4%)[8], we therefore plan to include 2,500 patients with a prehospital putative stroke. Lost to follow-up rate is estimated to be less 5%. There is no planned replacement of patients lost to follow-up.

The primary endpoint analysis will be performed on the ***per-protocol population***.

Interim analysis

We will perform an interim analysis for evaluation of the actual event rate after inclusion of 50% of the patients. If this shows a lower good outcome rate than expected, a new sample size calculation will be performed. Furthermore, the Data monitoring committee (DMC) will perform an independent safety analysis and will review the overall safety and efficacy according to the DMC charter (planned in the early phase of the trial, at 12 month and hereafter at least every 18. months) An additional safety interim analysis of mortality and hematoma expansion in ICH patients will be performed. Interim analysis are assessed using a level of significance of 1%.

The trial can be stopped at interim analyses for both futility, efficacy, and safety reasons.

Detailed description is found in the DMC charter.

Missing data:

Missing data with be handled using multiple imputation, in which missing cases are first filled in by several sets of plausible values to create multiple completed datasets and then the multiple sets of results are combined to yield a single inference.

Deviations from the statistical plan:

All deviations from the statistical plan will first be applied when approved by the regional ethics committee and the Danish Medicines Agency.

Trial decision making and stopping rules

A Data Monitoring Committee (DMC) will be appointed before trial start and include a signed DMC charter. The DMC will review the accumulated data during RESIST trial and provide advice on the conduct of the trial to the Steering Committee.

The DMC should inform the Steering Committee
if, in their view:

1. the results are likely to provide convincing evidence that one trial arm is clearly indicated or contraindicated, and there is a reasonable expectation that this new evidence would materially influence patient management

or

2. it is beyond doubt that no clear outcome would be obtained (futility)

It is not expected that RESIST trial will be stopped for reasons of efficacy or futility based on observed treatment differences. A predefined pooling of individual patient data (Remote Ischemic conditioning in Stroke Collaboration, RISC) is planned. To dispel scepticism, a robust demonstration of efficacy of this simple intervention is required. The primary reason to recommend stopping the trial will be for safety reasons.

For Acute Ischemic Stroke and Intracerebral Hemorrhage:

- For the primary endpoint the DMC will consider stopping if there is a robust reduction in prehospital stroke score (PreSS), for Acute Ischemic Stroke and Intracerebral Hemorrhage patients, during the first 24-hours in the sham (control) group compared to the intervention (RIC) group, achieving $p<0.01$.
    - A substantial number of patients experience serious adverse events ($p<0.01$)

- For the primary endpoint the DMC will consider stopping if there is an increase in mortality or stroke recurrence for Acute Ischemic Stroke and Intracerebral Hemorrhage patients in the intervention (RIC) group compared to the control (sham) group, achieving $p<0.01$.

- For the primary safety endpoint in ICH the DMC will consider stopping/modification if there is a clear evidence hematoma growth during the first 24-hour in the intervention group, achieving $p<0.01$

The DMC will make recommendations, which could include:

- No action needed, trial continues as planned
- Early stopping due, for example, to clear benefit or harm of a treatment, futility, or external evidence
- Stopping recruitment within a subgroup
- Extending recruitment (based on actual control arm response rates being different to predicted other than on emerging differences) or extending follow-up
- Stopping a single arm of a multi-arm trial
- Approving and/or proposing protocol changes