PROTOCOL

Version NO. 6 of 24/04/2017

Lower limb ischaemic preconditioning in the acute phase of cerebral infarction (<H6): a multicentre randomised study with clinical and MRI assessment.

RESCUE- BRAIN Study

(REmote iSchemic Conditioning in acUtE BRAin INfarction Study)

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LIST OF ABBREVIATIONS

ADC:	Apparent Diffusion Coefficient
ANSM:	French National Medicines Safety Agency
APTT:	Activated partial thromboplastin time
BI:	Brain Infarction
BP:	Blood pressure
CNIL:	French National Data Protection Commission
CONSORT:	CONsolidated Standards of Reporting Trials
CRA:	Clinical Research Associate
CRF:	Case Report Form
CT:	Computed tomography (CT scan)
DWI:	Diffusion-weighted Imaging (a diffusion-weighted image used in MRI)
EC:	Ethics Committee
IV:	Intravenous
RIC:	Remote ischaemic Conditioning
MA:	Marketing Authorisation
MRA:	Magnetic Resonance Angiography
MRI:	Magnetic Resonance Imaging
mRS:	Modified Rankin Scale (handicap score) (*Cf.* Annex 4.2)
NIHSS:	National Institute of Health Stroke Scale: clinical score of initial severity and prognosis (*Cf.* Annex 4.1)
RIPerC:	Remote ischaemic perconditioning
RIPreC:	Remote ischaemic preconditioning
rt-PA:	Actilyse ®
STAIR:	Stroke Therapy Academic Industry Roundtable
STIR:	Stroke Imaging Research Group
TIMI:	Thrombolysis In Myocardial Infarction (angiographic Re-canalisation score)

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RESCUE- BRAIN Study

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Introduction

Stroke represents the leading cause of acquired handicap and the second leading cause of dementia and death in so-called developed countries and throughout the world. [1-3] Brain infarction (BI) is the most common form of stroke (80%). The only approved treatment in the first 4½ hours is intravenous thrombolysis with rt-PA (Actilyse®), the aim of which is to recanalise the occluded artery and reperfuse the cerebral parenchyma. [4, 5] Few patients (1-5%) are treated because of the narrow time window, lack of knowledge of the warning signs of stroke in the general population, the painless and multiple nature of symptoms (primarily problems with language, motor deficit, sensory deficit, visual disturbance and dizziness) and the need for cerebral imaging to make the diagnosis of brain infarction, which lengthens the time between diagnosis and treatment.[6] Despite these treatments, 50 to 60% of patients still have a handicap at 3 months. [6, 7] This handicap correlates closely with the final size of the infarction.[8] To date, the neuroprotective treatments which reduce the size of the brain infarction in animal experiments have not been shown to reduce 3 month handicap in large randomised controlled trials.[9] Following an analysis of these failures, guidelines have been issued by working groups including the STAIR (Stroke Treatment Academy Industry Roundtable) and STIR (STroke Imaging Research group) groups. [10-12] We have taken account of these guidelines in the design and methodology of our study.

Remote ischaemic perconditioning (RIPerC) has been shown to have a neuroprotective effect in animal models of cerebral ischaemia by reducing the final size of the brain infarction.[13] In the case of brain ischaemia, RIPerC consists of iterative ischaemia in a limb with an electronic tourniquet or pressure cuff. RIPerC has recently been shown to have a cardioprotective effect in patients in one randomised study which included 250 patients during the first 6 hours following a myocardial infarction and who were candidates for primary angioplasty. [14] In this study, as in the other studies in patients, no significant side effects of RIPerC were observed. The mechanisms of action of RIPerC are partially understood and probably involve systemic release of endocrine factors which protect against reperfusion damage.

As regard, the interest of RIPerC in the hyperacute phase of brain infarction (<6H), a single-centre Danish study was recently published in November 2013. [15] [16] There are major methodological differences between our multicentre study and the single-centre Danish study, the latter was negative for the primary outcome.

1. Background and current state of knowledge

1.1 Stroke a public health issue

Each year, 150,000 new patients suffer a stroke in France. Stroke is the leading cause of acquired motor handicap and the 2nd leading cause of dementia and death throughout the world.[1-3] Brain infarction (BI) constitutes 80% of stroke and cerebral haemorrhage accounts for 20%. In both of these forms of stroke, stroke centers (SC) have shown a modest reduction in morbidity and mortality of 20%.[6] Optimally, all patients who suffer a stroke should be treated in a SC , although this is unfortunately not the case in the majority of countries.[6]

In brain infarction, the only approved treatment in the first 4½ hours (H4.5) is intravenous thrombolysis, rt-PA (Actilyse®). The aim of this treatment is to recanalise the occluded artery and reperfuse the distal cerebral parenchyma.[4-6] The clinical benefit of this treatment has been demonstrated in 2 pivotal studies by an increase in the percentage of independent patients at 3 months (Rankin score between 0 and 1; *cf.* Annex 1.2). In the 1st study, which was published in 1995, the percentage of independent patients was 40% in the group treated in the first 3 hours with rt-PA compared to 25% in the placebo group.[4] In the second study which was published in 2008 and included patients between H3 and H4.5, 52% of patients became independent in the group treated with rt-PA compared to 45% in the placebo group.[5] No excess mortality associated with rt-PA was observed in either study. Although this treatment represents a major therapeutic advance, few patients benefit from it (1-5%) for the reasons described in the introduction, and 50 to 60% of patients treated still have a handicap at 3 month. [7] This handicap correlates closely with the final size of the BI.[8] Therefore, in addition to stroke center and rt-PA, there is a need to develop other treatment approaches in order to reduce the morbidity and mortality associated with brain infarctions. [6] Neuroprotective treatments are candidates to reduce the final size of the BI, and usually have a large target population.

1.2 Brain ischaemia: a dynamic and potentially reversible process

Cerebral ischaemia is a dynamic phenomenon (which progressed rapidly over time, particularly during the initial minutes and hours) and is, in part, potentially reversible, particularly with re-canalisation. [17, 18] During arterial occlusion, cerebral perfusion studies have distinguished 3 areas distal to the occlusion, which have different metabolic pattern (*cf.* Figure 1) [17-19]:

- The core of the ischaemic site = brain infarction = irreversible ischaemia. This area becomes necrotic regardless of the revascularisation or neuroprotection techniques used.
- The area around the infarction = ischaemic penumbra = potentially reversible ischaemia area particularly if the blood flow is re-established (reperfusion). The ischaemic penumbra is the target for re-canalisation and neuroprotection methods.
- The area remote to the penumbra = oligaemia in which the blood flow is below normal but does not threaten progression to necrosis.

Each patient is in a different pathophysiological situation. Multimodal brain MRI is used to assess the area of infarction (hypersignal on a diffusion-weighted image) and the penumbra area (either using a perfusion image or with apparent coefficient diffusion (ADC) mapping)[19, 20]. The target of treatment, so called the mismatch, is the brain area in the ischaemic penumbra which is not yet infarcted (*cf.* Figure 1).

The main mechanisms of the growth of a brain infarction in the acute phase is the transformation of the ischaemic penumbra area into an infarction. Factors that may promote growth of the infarction area at the expanse of penumbra area, include : fever, hyperglycaemia, low systemic arterial blood pressure and reperfusion lesions[6]. Conversely, IV rt-PA and the neuroprotective treatments act to limit the growth of the area of infarction. Several models or processes which are considered to be neuroprotective have been shown to be effective in reducing the final size of the infarction in animal experiments but to date none has shown a benefit in terms of functional outcome in patients in large randomised controlled trials.[9]

1.3 Neuroprotection in patients with brain infarction

Recent reviews have identified main reasons that explained the bench to bedside translation failure of potentially neuroprotective drugs or interventions. [9, 21] Based on these analysis the Stroke Treatment Academic Industry Roundtable (STAIR) working group have produced guidelines in 1999 and then in 2009.[10-12] The main reasons for these failures in this translational research have included:

- Inadequate methodological quality of the animal studies: no randomisation, no blind assessment of the outcome, effect tested on a single species of animal and no studies on "sick" animal models. These sources of bias overestimate the neuroprotective effect of the molecule or intervention. Since the first publication of the STAIR guidelines, the methodological quality of the preclinical studies has improved and some journals require these criteria as prerequisites to allow publication. The preclinical studies on the neuroprotective effect of ischaemic conditioning which have recently been studied do not contain these sources of bias.
- The neuroprotective treatment administration time window used in patients is too wide: many studies in patients have tested neuroprotective treatments during the first 12 or 24 hours following the brain infarction. [21] This time window is too wide as the experimental findings in patients and in animals indicate that a time window of the first 6 hours following the brain infarction should be recommended. [9, 21]
- Use of different endpoints: tissue in animals (reduction in brain infarction size); and clinical in patients (functional outcome at 3 months).

The primary endpoint in neuroprotection trials in patients have been functional outcome, particularly the Rankin score at 3 months, i.e., the same outcome as in the pivotal studies on rt-PA. In order to demonstrate clinical benefit of a neuroprotector using this score, between 1,000 and 3,000 patients need to be included. [22] The last 4 large neuroprotection studies with clinical outcome included between 1,000 and 3,000 patients and were all negative: CHIMES (n=1100); SAINT 2 (n = 3306); CASTA (n=1070); ICTUS (n=2296).

In view of these data, working groups (STAIR, STIR) [10-12] recommend assessment of the neuroprotective effect in patients following 2 steps:

- First step is to demonstrate that the neuroprotective treatment reduces the volume of the brain infarction in patients. The advantages of using the volume of brain infarction as an outcome compared to the clinical criterion are as follows:
  - The number of patients which needs to be included is reduced by a factor of 10 [23] and involves between 100 and 300 patients.
  - It is the same outcome as is used in animal experiments
  - It is a direct objective measurement and is a biomarker. Handicap correlates closely with the final size of the infarction [23, 24] but represent a different level of complexity in which many other factors are involved including patient age, comorbidities, social and family environment and access to rehabilitation.[24]
- Second step: if the neuroprotective effect is confirmed in a proof of concept study with patients (through a reduction in the volume of the brain infarction in the neuroprotection group compared to the control group), a large randomized controlled trial using functional outcome primary endpoint (i.e. 3-month Rankin scale) could be carried out. Calculation of the number of patients required will be optimised from findings from the initial study.

Our study represents the first step recommended by the working groups.

1.4 Ischaemic conditioning

a) Definitions

Ischaemic conditioning consists of period of transient ischaemia in a tissue or organ before, during or after the onset of prolonged ischaemia (usually myocardial or brain infarction). This transient ischaemia is believed to promote the release of endogenous cytoprotective factors which reduce the final size of the infarction in the target organ.[25] Depending on when the transient ischaemic stimuli are applied regarding the prolonged ischaemia, the following can be distinguished:

- Pre-conditioning: several episodes of transient ischaemia before prolonged ischaemia
- Per-conditioning: several episodes of transient ischaemia during prolonged ischaemia when the artery is still occluded
- Post-conditioning: several episodes of transient ischaemia after re-opening of the artery responsible.

Conditioning can also be carried out on the organ itself (“conventional’’) or remote from the organ (“remote”)

b) Mechanisms involved in ischaemic conditioning

The mechanisms involved in ischaemic conditioning, particularly remote conditioning, are not entirely understood. [13] Experimental studies have shown involvement of different signals and pathways arising from the remotely conditioned tissue. Three pathways appeared to be involved between the remotely conditioned tissue and the target organ: the blood circulation, the autonomic nervous system and the white blood cells. [13] Figures 3 and 4 in the annex summarise the mechanisms involved. The protective effect is not dependent on any one species and can be transferred from one animal to another via plasma if the autonomic nervous system is disease-free. [26] The mediators have not been completely identified. Some studies suggest a role of autacoids such as adenosine, opioids and bradykinins which may either pass into the blood circulation or stimulate the vagal nerve (anti-inflammatory cholinergic system).[13] Remote ischaemic conditioning in the target organ activates a common pathway through the release of intracellular kinases (‘survival kinases’) and results in opening of the

ATP-K^+ dependant channel and closure of the permeability pore in the mitochondria which protects the cell from reperfusion lesions and cell death. [13] Finally, in terms of the neuroprotective effect, Remote ischaemic preconditioning (RIPreC) improves cerebral blood flow which may also play a role in the neuroprotection which is seen in animals.[13]

c) History and ischaemic conditioning in cardiology

During the 1980s, the benefit of ischaemic conditioning was demonstrated in myocardial infarction (MI) in animals. In 1986, Murry showed in dogs that brief episodes of myocardial ischaemia prior to more prolonged ischaemia reduced the final size of the MI [27]. This endogenous protective effect is called ischaemic preconditioning (RIPreC). In clinical practice this represents the clinical setting of episodes of angina prior to the MI. It has been found in large clinical studies that these patients have smaller MI and fewer complications. [28] A few studies have suggested similar results in neurology with less severe brain infarctions when these are preceded by transient ischaemic attacks ("TIA"). [29, 30]

i) Interventional Cardiology

Interest in this subject has returned with the demonstration of a cardioprotective effect of ischaemic post-conditioning in MI in animal experiments. [31] This represents the clinical situation of the interventional cardiologist during a primary angioplasty after re-opening of the culprit artery. One randomised trial has assessed the benefit of four 1-minute inflation cycles of the angioplasty balloon one minute after reopening the affected coronary artery and stenting. [32] These cycles were separated by a period of one minute deflation. The 30 patients had a MI which was less than 6h from onset and all underwent primary angioplasty. The group which received post-conditioning in addition of usual care had an area under the curve of cardiac enzymes reduced by 36% in comparison to the control group. [32] These results were confirmed at 6 months and 1 year with myocardial scintigraphy and echocardiography.[33]

ii) Planned cardiac surgery and coronary angiography.

Remote ischaemic preconditioning (RIPreC), involving a cycle of transient ischaemia in a limb is more easy to use in clinical practice and represents a turning point in the field of ischaemic conditioning, now that its cardioprotective effect has been shown in animal experiments.[34] One randomised study that included 57 patients and published in the *Lancet* demonstrated the benefit of RIPreC in patients undergoing coronary artery bypass surgery. [35] RIPreC carried out after general anaesthesia and before clamping the aorta, involved three 5-minute cycles of inflating an arm cuff to 200 mmHg with a 5-minute deflation period between each cycle. Peroperative myocardial suffering assessed by the area under the curve of repeated troponin measurements was lower in the RIPerC group. This beneficial effect found initially on cardiac biomarkers has been confirmed with "hard" clinical criteria including mortality. [36] Eighteen-month mortality rates in 329 patients undergoing coronary artery bypass surgery randomised in the RIPreC or control group and the development of a major cardiovascular event were reduced significantly in the RIPreC group (1.9% compared to 6 .9%, p=0.046; 13.9% compared to 18.9%; p<0.01). [36] Similarly, one English randomised study on 244 patients has shown that RIPreC prior to planned coronary angiography reduced the H24 troponin peak and also reduced major cardiovascular events at 6 months. [37] The same group has shown that this benefit was maintained at 6 years. [38]

Two other randomised controlled trial in planned cardiac surgery have shown a cardioprotective effect of RIPreC. An initial study which included 81 elderly patients who underwent valve replacement surgery [39] and a second study in 37 children, average age one year old, who underwent surgery for congenital heart disease. [40] In these situations, the RIPreC was applied to the lower limb.

iii) Myocardial infarction

A randomised controlled single centre Danish trial has shown the benefit of remote ischaemic perconditioning (RIPerC) before primary angioplasty in patients during the acute phase of a myocardial infarction. [14] As the diagnosis of MI can be made in prehospital setting,

the patients underwent either a RIPerC procedure or received usual care prior to their arrival in hospital for primary angioplasty. The primary outcome was a biomarker, the salvaged myocardial tissue index as assessed by SPECT.[14] This was assessed blind to the RIPerC or control group. The study included 250 patients and was positive for the primary outcome. A large multi-national multicentre study involving 2,300 patients which is ongoing in order to assess the effect of RIPerC prior to primary angioplasty in the acute phase of an MI on clinical endpoints (cardiovascular mortality and hospitalisation for heart failure at 1 year). (CONDI2 study. Website: http://clinicaltrials.gov/; study reference: NCT01857414). This approach used in MI: the first study on a biomarker and based on these results pursue with of a large randomised controlled trial with clinical primary endpoint illustrates the approach recommended for the neuroprotection studies that we aim to follow.

1.5 Neuroprotection and ischaemic conditioning

a) Animal experimentation

Demonstration of the protective effect of ischaemic conditioning in animals is more recent.[41] Following ligation of the carotid artery or middle cerebral artery in the rat, different preconditioning protocols have been shown to have a neuroprotective effect. [41] RIPerC was more effective than RIPreC in reducing the final size of the brain infarction in one study in the rat following arterial ligation. [42] In order to come closer to patients with BI, RIPerC was tested in a model of embolic brain infarction (which is different from the mechanical model of arterial ligation) and either treated or not treated with rt-PA. RIPerC was effective in reducing brain infarction size and had an additive effect with rt-PA.[43] Preclinical studies have followed the STAIR group guidelines, particularly in terms of randomisation, measurement of outcome blind to the allocation group, the use of different RIPerC protocols, experiments on different species of animals and in models of animals with comorbidities.[10, 11]

b) Studies in patients

Two studies have been conducted in neurovascular disease in patients in different contexts of the acute phase of brain infarction. The first study assessed the feasibility and tolerability of

RIPerC in a cohort of patients with aneurysmal sub-arachnoid haemorrhage (SAH). One of the complications of SAH is vasospasm which causes a brain infarction. Thirty-three patients received RIPreC with 3 repeated ischaemic cycles with a cuff inflated to 200 mmHg for 5 minutes followed by 5-minute periods with the cuff deflated, either in an arm or in a lower limb (the cycles in these cases lasted 7.5 or 10 minutes). [44] RIPerC in the arm and in the leg was well tolerated with no complications or cases of discontinuation because of patient discomfort.

The second study was an open label randomised study in patients with symptomatic intracranial stenosis.[45] All of the have patients received secondary preventive medical treatment and the RIPreC group received in addition five cycles of 5 minutes of transient ischaemia with 5 minutes of the cuff deflated between cycles twice daily for 300 days simultaneously in both arms. The device involved 2 electronic tourniquets connected through the same console. The RIPreC group had a significantly lower cerebral ischaemia recurrence rate at M1 and M 10 ($p < 0.01$). Cerebral perfusion assessed by SPECT was improved in the RIPreC group. [45]

Both of these studies provide reassuring safety findings for remote ischaemic preconditioning in patients with cerebrovascular disease and support the cardiology safety data. We should note that the electronic tourniquet is used daily in the operating room for upper or lower limb orthopaedic surgery in order to obtain an "exsanguinated" operating area (because of the cessation of arterial and venous blood flow). It can also be used during loco-regional anaesthesia. [46] In these situations, the tourniquet duration involve a continuous period of 1h to 2h. A tourniquet time of over 2 hours is not recommended. [46] In the Norwegian national surveillance system of 65,000 surgical procedures in which a tourniquet was used, a complication was found in 26 cases (4/10,000), 2 of which were permanent. [47] These were neuronal lesions which occurred during surgery with a tourniquet duration of over 3 hours. The first involved the upper limb and resulted in a permanent radial nerve injury when the patient had had a tourniquet applied for 2 hours 10 min at a pressure of 300 mmHg to the arm. The second involved external popliteal nerve injury following a thigh tourniquet applied at 250 mmHg for 3h. We are intending in our cycle to administer 4 cycles of 5 minute tourniquet time

to the thigh at a pressure of 110 mmHg above the systolic arterial blood pressure, which is a common situation.[47]

In terms of the effect of RIPerC during the acute phase of brain infarction, a Danish study has just been published in November 2013 [15, 16] and was a single centre study. One hundred and twenty patients were included although only a minority received the whole RIPerC protocol. There were common points and methodological differences between the Danish study and our own:

- the single centre nature of the Danish study and multicentre nature of RESCUE BRAIN,
- the RIPerC procedure was performed in pre-hospital setting in patients with suspected brain infarction in the Danish study. In our study the RIPerC will be performed after the first MRI, and diagnostic certainty for BI. We note that unfortunately in the Danish study, ultimately only 33 patients (13%) out of the 247 patients included in the pre-hospitalisation RIPerC group had actually suffered a brain infarction and received the full RIPerC protocol with 4 cycles of cuff inflation and deflation. The design of RESCUE BRAIN involves inclusion after MRI (and therefore a diagnostic certainty of brain infarction) and the RIPerC protocol to be carried out in an stroke center enabling far more patients with a BI to be recruited and to undergo the complete RIPerC protocol.
- Both studies used changes in volume of the infarction on brain MRI as outcome. The volume of brain infarction is therefore used as a biomarker in both studies. There are differences between the MRI parameters used and in the time until the second MRI which are explained in section 3.1.
- The Danish study only included patients treated with IV rt-PA. In our study we are including all victims of a brain infarction within the first 6 hours. Animal studies have shown that RIPerC has been effective alone or with rt-PA.[43] Between 50 and 75% of patients in whom a thrombolysis pre notification is triggered ultimately do not undergo thrombolysis because of a contraindication to rt-PA. Being able to offer a treatment which improves the functional prognosis in these patients is therefore a major challenge.

2. Objectives of the trial

2.1 Hypothesis tested

Iterative lower limb ischaemia has a neuroprotective effect (reduction in final size of the infarct on D1) during the first 6 hours of brain ischaemia, whether or not the patient is treated with intravenous thrombolysis.

2.2 Main objective:

To demonstrate that ischaemic per-conditioning (RIPerC) to a leg in the first 6 hours of a brain infarction reduces brain infarction volume at H24, compared to the control group.

2.3 Secondary objectives:

- MRI: relative change in brain infarction volume defined by (Vol D1- Vol D0)/Vol D0 in the RIPerC group compared to the control group.
- Clinical efficacy: Change in the NIHSS score between D1 and D0, M3 Barthel and modified Rankin score.
- Re-canalisation rate in both groups whether or not they receive IV thrombolysis.
- Safety data:
    - haemorrhagic transformation rate in cases of IV thrombolysis
    - early neurological deterioration rate
    - death rate
- Tolerability and side effects of RIPerC

3. Design and selection of patients

3.1 Choice of experimental plan:

This is an interventional trial with a medical device, an electronic tourniquet used in common practice in the operating theatre.

The study design is an open-label randomised multicentre trial on parallel arms with blind assessment of the outcome (prospective randomised open trial with blinded end-point: PROBE design). Randomisation will be stratified on whether IV thrombolysis is given, as this is a major prognostic factor, and on the centres.

The 2 stratified thrombolysis study arms based on whether or not IV thrombolysis is given are:

Without RIPerC: usual patient care (thrombolysed or otherwise).

With RIPerC: usual patient care (thrombolysed or otherwise) with remote ischaemic per-conditioning.

Infarction volumes will be measured without knowledge of the time of the measurement (D0 or D1) or of the treatments received. The MRI CDs will be read on a centralised basis in the Versailles Hospital Centre Neurology Department.

a) Justification for the MRI endpoint

In section 1.3 concerning neuroprotection in patients, we justified the choice of the MRI outcome compared to the usual clinical outcome (percentage of patients with Rankin score <2 at 3 months) for our study.

The choice of our MRI procedure takes account of the STIR guidelines, i.e., simple, rapid, reproducible image acquisition sequences used in common practice and not delaying institution of treatments. [12] This is the reason why brain infarction volume is assessed by diffusion-weighted imaging which is the key image acquired in all centres in cases of suspected brain ischaemia. We are not using a perfusion weighted image or the concept of diffusion-perfusion

mismatch which was used in the Danish study as this requires injection of contrast medium and the 10 centres do not perform this routinely. In addition, this significantly prolongs the duration of the MRI and interpretation of the perfusion images is not standardised. [20, 47] In order to reduce the number of missing data points for the primary outcome (STIR guidelines [12]), the final volume used in our study is the diffusion-weighted volume on D1. It has been shown that measurement of this volume on D1 correlates closely with functional prognosis at 3 months. [48] We have chosen the difference in volume between D0 and D1 and not D5 as this reduces patients lost to follow-up and helps to avoid over-estimation of volume seen on D5 because of the maximum vasogenic oedema which is seen between D3 and D5. [20] This MRI protocol has already been used by one of the participating centres (Salpêtrière NVU, Prof Samson and Dr Rosso) in a randomised therapeutic trial in patients in the first 6 hours of a brain infarction. [49]

c) Independent monitoring committee (Data and Safety Monitoring Board- DSMB). All of the findings from clinical studies on RIPreC are reassuring as no local side effects of intense pain, thrombosis of the limb involved or ischaemia had no systemic effects (no increase in arterial blood pressure or heart rate) had been seen. However, in view of the profile of patients included in this study, brain infarction patients some of whom treated by IV thrombolysis, an independent data and safety monitoring board (DSMB) will be set up in order to ensure that no serious local complications occur and that the haemorrhagic transformation rate are the same in both groups.

3.2 Selection criteria:

- Inclusion criteria: in order to be eligible, the patients must meet all of the following criteria:

- Patients ≥ 18 years old
- Carotid brain infarction
- NIHSS between 5 and 25
- Brain MRI performed within 6 hours from the onset of symptoms
- Patients' written informed consent or consent from a third party (relative or family circle) in a non-urgent situation.

- Affiliation to a health insurance system (beneficiary or a person with rights from such a system)

- Non-inclusion criteria: in order to be included the patients must not have any of the following criteria:

- Patient in whom dual IV thrombolysis is planned
- The presence of a leg ulcer or poor lower limb skin state
- Past history of obstructive lower limb arterial disease
- Known sickle cell anaemia (risk of vaso-occlusive crisis)
- Past history of lower limb deep vein thrombosis
- Past history of brain infarction within 3 months
- Participation in another acute phase interventional study
- Patients under legal guardianship or trusteeship
- Diseases which are life-threatening within 6 months and make the 3-month assessment impossible
- Patients who were not independent prior to the BRAIN infarction (prior Rankin score of > 2)
- Pregnant women

3.3 Recruitment methods:

Patients will be recruited in 10 participating medium and high volumes stroke centres. Each centre has:

- a neurovascular intensive care unit (NVICU) which is independent (not located in a multidisciplinary intensive care unit or a cardiac intensive care unit)
- in situ on call

- 24-hour access to brain MRI.

Candidate patients for the RESCUE BRAIN study are those in whom a thrombolysis alert is triggered (any neurological deficit within 4 hours) who have had an initial brain MRI for this reason on an urgent basis. Details of the feasibility and activity of each of the 10 centres is given in section 3. The activity of each stroke center can be described by indicators ranging from the most overall, activity, to the more specific. As an example, for the Versailles Stroke center in 2012, 700 patients suffering from a stroke were treated, 385 of whom had an established brain infarction including 200 who were alerts for thrombolysis and 65 who actually underwent thrombolysis. The thrombolysis rate therefore accounts for 9% of all stroke, 17% of brain infarctions and 33% of thrombolysis alerts. In RESCUE BRAIN study, each centre is asked to include 20 patients/year for 2 years which represents between 4% and 10% of the thrombolysis alerts depending on the centre (*cf.* section 3).

3.4 Number of patients to be included and justification. Total N = 200, i.e., 100 in each group.

To calculate the number of subjects required we used firstly data from the literature on the growth of brain infarction volumes and in addition had access to individual data from a single centre trial set up by the Salpêtrière cerebrovascular emergencies group (Dr C Rosso, Prof Y Samson) which is taking part in our study.

a) Data from the literature on brain infarction growth volumes

Several MRI parameters can be used as an endpoint for neuroprotection studies. [22] We have opted for a difference in absolute rather than relative volume as there is a large variability in brain infarction volumes and in their change. A patient with an initial volume of 1 cm^3 which increases to 3 cm^3 on D1 has a relative difference of +300% although this is very likely to have no clinical impact. On the other hand, various authors have quantified changes in brain infarction growth volumes caused by factors known to be of clinical prognostic value such as re-canalisation or hyperglycaemia. In one study which included 100 patients with brain infarction

two-thirds of whom were treated with IV rt-PA IV, the mean volume of brain tissue salvaged (D1-D0) by re-canalisation of a middle cerebral artery was found to be 24 cm^3.[50] Conversely, patients who were hyperglycaemic on admission had a 29 cm^3 increase in brain infarction volume compared to normoglycaemic patients. [50] We should note that the volume of "salvaged" brain tissue from IV thrombolysis has not been assessed in the pivot studies in which patients were included on the basis of a CT scan.[4, 5] Another study which included 170 patients found a difference in volume of brain infarction (D5-D0) that of over 10 cm^3 associated with a poorer 3 month prognosis.[51]

b) Individual data from the INSULINFARCT study.

This study included 188 patients during the first 6 hours of a brain infarction.[49] The study compared 2 insulin therapy protocols to treat the hyperglycaemia during the acute phase: subcutaneous versus IV through an electronic syringe based on clinical and MRI criteria. Brain infarction growth volumes were assessed by MRI using the Neurinfarct© software. Using data from the SC insulin treatment group from the INSULINFARCT study, a change in infarction volume (infarction growth or IG) between D0 and D1 was distributed as follows: median 10.7, mean 27.1, standard deviation 35.9 cm^3. The mean difference between the two arms was around 15 cm^3. The percentage of patients who received IV thrombolysis was the same in both groups, at approximately 75%.

Based on these findings and on Prof Samson's personal data we consider that a minimal clinically relevant effect would be an average difference in increase in volume (D1-D0) of 15 cm^3 between the 2 groups. Assuming a probability threshold of 5%, a study power of 80% and a standard deviation of 36 cm^3, 92 patients are required per group, i.e., a total of 184 patients.[52] Assuming an additional 10% of subjects (because of unusable MRI), the total number of patients required is **200**.

This number is consistent with the numbers proposed by the MR Stroke Collaborative Group for neuroprotection studies using brain infarction volumes on MRI as the primary outcome. [22]

An interim analysis is intended after the first 102 patients had been included according to the Posch and Bauer procedure.[53] At the end of this interim analysis the independent monitoring board will decide whether or not the study is to be continued taking account of the efficacy and tolerability (incidence of SAE) data in both groups.

3.5 Duration of participation for each person:

Each patient will be followed up in the study for 3 months.

3.6 Randomisation and measures to reduce bias:

Patient randomisation will be centralised (on the web) and will be based on a list which is pre-determined by a computer programme and confirmed according to the sponsor's procedures. Randomisation will be on a ratio of 1:1, stratified by centre and matched by variable or invariable size block indicated. To check for any selection bias, a register of eligible non-included patients will be held in each centre showing the date of eligibility and any reason for non-inclusion. This register will be cross-compared with the centre's PMSI data.

The primary outcome will be assessed on the basis of an objective criterion blind to treatment arm in order to avoid measurement bias.

4. Plan and conduct of the trial

4.1 Patient follow-up:

a) Chronology and content of visits

- Inclusion:

- Socio-demographic data
- Vascular risk factors (hypertension, smoking habit, diabetes, hypercholesterolaemia, permanent atrial fibrillation, heart failure and physical activity)
- Past medical history (past history of TIA or CI)
- Treatments on admission
- Score: NIHSS (Annex 1)
- Rankin score (Annex 2)
- Vital signs: blood pressure, heart rate, temperature
- BM, venous blood glucose
- Initial brain MRI
- Decision to thrombolyse with IV rt-PA
- Consent from the patient or family in a non-urgent situation.
- Randomisation stratified by centre and by IV thrombolysis
- Conduct of the RIPerC protocol
- Tolerability of the RIPerC: pain and local complications.

- D1:

- Score: NIHSS (Annex 1)
- Vital signs: blood pressure, heart rate, temperature
- Current and ongoing treatments
- Repeat brain MRI (between H24 and H36 after the D0 brain MRI)

- D7 (+/- 2 days):

➢ Score: NIHSS (Annex 1)

➢ Vital signs: blood pressure, heart rate, temperature

➢ Ongoing treatments

- 3 months (+/- 10 days):

➢ Rankin and Barthel scores (Annex 2)

➢ Score: NIHSS (Annex 1)

➢ Vital signs: blood pressure, heart rate, temperature

➢ Ongoing treatments

b) Procedures, investigations and samples

Functional scores:

The functional scores used in this study originate from scales which are conventionally used and have been validated internationally to assess patients who have suffered a brain infarction. These are used in common practice by doctors in the participating centres at the same frequency as is described for the study. These scales are shown in the annex:

The NIHSS score (Annex 1) is used mostly during the acute phase and quantifies the neurological deficit in a standardised manner with 11 manoeuvres. It takes on average 7 minutes to complete and offers excellent reproducibility. The score ranges from 0 (no deficit) to 42 points. Intravenous thrombolysis is delivered classically for an NIHSS score of between 4 and 25. The 3 month prognosis correlates closely with the initial NIHSS score.[6]

The Rankin score (Annex 2) is a score used for patient monitoring. This assesses functional handicap and ranges from 0 (patient without symptoms) to 6 (death). The randomised controlled trials on brain infarction acute treatment have often used a comparison between the percentage of patients with a Rankin score of between 0 and 1 as the primary endpoint in the 2 groups (patients who are independent in activities of everyday living).[4, 5]

The Barthel score is the score which assesses patient independence in activities of daily living. This is scored from 0 (the patient is dependent for all activities of daily living) to 100. The percentage of patients with a score of ≥ 95 at 3 months in each of the groups is a secondary outcome which is classically used in acute phase studies.[4, 5]

BP: Blood pressure will be taken in both arms before starting the RIPerC protocol (to investigate for asymmetry of blood pressure in 2 arms suggesting aortic dissection presenting with neurological signs or subclavian artery stenosis)

Blood samples: the blood profile will be the profile which is conventionally performed with a full blood count, platelets, PR, APTT, blood electrolytes, urea, creatinine, CRP, blood glucose, glycated haemoglobin, LDL, HDL and total cholesterol and triglycerides. Admission blood glucose measurements will be used in our study as the adjustment variable in view of its prognostic value. We have not planned to create a plasma archive for financial reasons and because of the fact that the neuroprotective effect of RIPerC has not been demonstrated in patients. If our study is positive, a larger study will be conducted and an ancillary laboratory study will then be organised.

Brain MRI:

The initial and repeat brain MRI will be performed in each of the centres. The protocol used in each centre involves diffusion-weighted imaging (DWI), FLAIR imaging, a T2 star image and a 3D-TOF circle of Willis MRA. In short, the indices obtained from the FLAIR image are: 5 mm thick axial sections (1.5 mm between sections), 256x256 matrix, Repetition Time (RT) = 8,800 ms, Echo Time (ET) = 140 ms, Inversion Time (IT) = 2,200 ms, image acquisition duration = 2 min 56 sec.

The diffusion-weighted image involves 24 x 5 mm thick sections (0.5 mm inter slice) 96x64 pixel matrix, ET=98.9 ms and RT = 2,825 ms. A standard image acquisition (b=0) and an image acquisition with a diffusion gradient of b=1,000 s/mm^2 will be performed over 40 seconds.

The 3D-TOF MRI has the following parameters: 1.4 mm section thickness, matrix 256x192, RT=2,825 ms, ET= 92.6 ms, duration=2 min 39 sec.

The star T2 image is an image acquired over 1 minute with 6 mm axial sections, ET =15 ms, RT=500ms, and angle=20°.

4.2 Study drop-outs and discontinuation of the study

Any patient may be withdrawn from the study:

- at his/her request. The reason for dropping out of the study will be recorded wherever possible and the patient's agreement will be confirmed in order to use data which have already been collected.
- by discontinuation of their participation on the decision of the investigator.

The study may be stopped temporarily or permanently:

- On a decision by the co-ordinating investigator with the agreement of the Scientific Committee which is set up and by the study sponsor.
- On the opinion of the independent study safety board.
- If new scientific data are published which may cast doubt on the research.
- If findings come to knowledge which compromise the study being conducted for patient safety reasons.
- In the event of inadequate recruitment according to the terms set by the sponsor.

5. Medicinal products, medical devices

The per-conditioning protocol will use an automatic electronic tourniquet. The specifications are for a regulated 1 pressure circuit electronic pneumatic tourniquet model with an integral printer and rolling casters. The Dessillons & Dutrillaux electronic tourniquet, Reference type G10802 has been chosen (see-below).

5.1 Summary of product characteristics

D&D
Depuis 1949

CE 0120

GARROT PNEUMATIQUE ELECTRONIQUE A 1 CIRCUIT DE PRESSION REGULEE

Référence: G10802

-Ce dispositif géré par microprocesseur est une garantie de sécurité, de précision et de traçabilité
-Le réglage de la pression et du timer ainsi que la décompression temporaire est très simple grâce au clavier en façade
-Le clavier alphanumérique permet la saisie d'informations destinées à la traçabilté : patient, hôpital, le système est horodaté;
Informations exploitablent via une imprimante et / ou sur la connexion USB.
-Une batterie assure l'autonomie du dispositif
-Des alarmes sonores et visuelles assurent la sécurité du dispositif
-L'interface de commande est très faciler à nettoyer,
Le clavier est traité antibactérien et la façade en inox

-A10701:
Pied mobile à roulettes
avec panier

-G13100:
Imprimante intégrée

CARACTERISTIQUES TECHNIQUES:

CONDITIONS DE STOKAGE	0 à 50°C/95% Taux d'humidité
CONDITIONS D UTILISATION	0 à 40°C / 95% Taux d'humidité
DIMENSIONS	
Hauteur	340 mm
Largeur	300 mm
Profondeur	180 mm
POIDS	5,6 Kg
ALIMENTATION ELECTRIQUE	
Tension	100- 240 VAC
Fréquence	50-60 Hz
Puissance	30VA
CLASSE ELECTRIQUE	I
BATTERIE	NIMH / 12 V / 4000 Ma
Alarme défauts	Sonore et visuelle
PRESSION	
Unité	mmHg
De service	1,7 bar
Plage de réglage	0 à 600 mmHg
Précision de réglage	1 mmHg
Précision de pression	.+ - 5 mmHg
Circuit de pression	1
Connection pneumatique	1
Alarme défauts	Sonore et visuelle
MINUTERIE	
Unités	Secondes et minutes
Alarme	Sonore et visuelle
NIVEAU SONORE MAXIMAL EN FONCTIONNEMENT	56Db

Dessillons & Dutrillaux Zone Industrielle La Tuque 47240 Castelculier
Tél: 05 53 48 30 66 Fax: 05 53 47 24 64 Email: ventes@ddmedical.fr
web site: www.ddmedical.fr

Certifiée ISO 13485 : 2003 par SGS
Certificat ISO FR03/59490
Certificat CE FR03/59489

8

5.2 The conditioning protocol used in our study:

- The conditioning protocol will be written by the Versailles Hospital Centre Mobile Clinical Research Group (EMIC) State Registered Nurses: Nadège Fleury & Cécile Mariet. The protocol will be presented, approved and training provided to the State Registered Nurses in the participating departments.

- It involves 4 inflation cycles of a lower limb thigh cuff at 110 mm Hg above systolic BP for 5 minutes. [47] The cuff inflation phases (transient ischaemia) will be separated by 5-minute periods in which the cuff is deflated. The protocol will be pre-programmed into the electronic tourniquet console. The total duration of the RIPerC protocol is 40 minutes (4 x 5-minute inflation phases and 4 x 5-minute deflation phases).

- The State Registered Nurse who delivers the RIPerC protocol must ensure that pedal or posterior pulses have disappeared during the 1st inflation cycle.

- The RIPerC protocol must be started within 6 hours of the onset of the brain infarction and as soon as possible after the brain MRI is performed. In patients who receive IV rt-PA IV thrombolysis on a decision made prior to randomisation the protocol will be carried out during thrombolysis.

- The cuff will be positioned in no specific situation on the opposite side to the motor deficit if one exists in order not to increase any risk of venous thrombosis.

- If the patient is randomised into the control group, the electronic tourniquet will be positioned in the same manner as above although will remain deflated for 40 minutes.

- A nurse monitoring form will be provided with:

  - Identification of the study and patient
  - BP before and at the end of the RIPerC procedure
  - Visual analogue scale for pain
  - A record of the 4 inflation/deflation cycles will then be printed and inserted into the case report form (CRF).

- A care worker will be present during the per-conditioning period to ensure that the machine operates correctly.

5.3 Data acquisition for the study

- As stated above, no acquisition of medical materials is intended for this trial. Ten Dessillons & Dutrillaux G10802 electronic tourniquets will be rented for the period of the study.

6. Treatment administered to people taking part in the research

6.1 Description of the treatment required to conduct the research

The lower limb RIPerC protocol has been conceived in order not to interfere with rt-PA (Actilyse®) thrombolysis which will be performed according to its MA and guidelines from the learned societies: 0.9 mg/kg with a bolus dose of 10% of the dose followed by 90% of the dose by IV electronic syringe with monitoring of arm blood pressure every 15 minutes.

6.2 Permitted and prohibited medicinal products and treatments in the study

There are no specific medical treatments for this study. The treatments which patients will receive are those which are usually prescribed in the acute phase of brain infarction consistent with international guidelines.[6]

These are primarily:

- IV thrombolysis with rt-PA (Actilyse®). When IV thrombolysis is given consistent with guidelines, no antiplatelet or anticoagulant treatment will be prescribed during the first 24 hours. Although at present there is no scientific evidence to support the benefit of endovascular treatment (thrombectomy or in situ thrombolysis) in addition to IV thrombolysis, this treatment is left to the decision of each investigating centre and is neither an exclusion criterion nor a criterion for being withdrawn from the study. Endovascular treatment and the type of treatment will be recorded in the case report form.
- Anti-thrombotic treatment:
    - Anti-platelet agents: aspirin, clopidogrel
    - Anticoagulant (preventative dose in the majority of cases): LMWH or UFN
- Analgesic treatments: paracetamol
- Antibiotic treatment if required: 3rd generation cephalosporin.

No treatments are prohibited in this study.

6.3 Treatment adherence monitoring methods.

Ongoing treatments on D0, D1, D7 and M3 will be recorded in the case report forms (CRF)

7. Outcome measurments

7.1 Variable measured for the primary outcome

The brain infarction volumes on D0 and D1 will be determined at the end of the study:

- blind to clinical findings, treatments received, date when the MRI was performed (D0 or D1) and patient identification, in order to avoid D0 and D1 being identified on a pair of MRI films.
- Lecture will be performed at the neurology Department and Stroke center, Versailles Hospital Centre,
- using a validated methodology already used in a previous clinical trial.[47, 54]

Volumes will be calculated using Neurinfarct© software (Intelligence in Medical Technology, Paris). This software calculates the volume in cm^3 from a semi-automated delineation (manual validation) of the clear hypersignal (the infarction) on a diffusion-weighted image (b value = 1,000 $s/mm^{2)}$.

7.2 Variables measured for the secondary outcome:

- Patient handicap at 3 months will be assessed through the Rankin score and the Barthel score (*cf.* annexes 1 and 2) during the 3-month visit.
- When the MRIs are read the following will also be scored:
  - The status of the intracranial arteries and presence of a stenosis or occlusion will be recorded.
  - If an arterial occlusion responsible for the brain infarction on the initial MRA is seen the TICI Re-canalisation score will be used in order to grade the level of re-canalisation.

- The presence of haemorrhagic transformation on a brain MRI on D1 will be recorded using the ECASS classification[5]. This incorporates 4 classes of increasing severity:

  - ***HI-1: Type 1 haemorrhagic infarction:*** small petechiae peripheral to the infarction
  - ***HI-2: Type 1 haemorrhagic infarction:*** confluent petechiae in the infarcted area with no mass effect
  - ***PH1: Type 1 parenchymal haematoma:*** haemorrhage ≤ 30% of the infarcted territory and mass effect
  - ***PH2: Type 2 parenchymal haematoma***: haemorrhage > 30% of the infarcted territory and mass effect.

This classification which was initially established on CT has been validated for MRI. [55, 56]

7.3 Study schedule: table

		D0		D1	D7	M3
		Admission	Before and after RIPerC			
Clinical data	Past medical history	●				
	CV risk factors	●				
	NIHSS	●		●	●	●
	Temp, Sa02, BM	●		●		
	BP, HR	●	●	●	●	●
	Rankin and Barthel scores	● (Rankin)				●
Laboratory data	Venous blood glucose	●				
Radiological examinations	Brain MRI	●		●		
Randomisation		●				

8. Safety assessment criteria

8.1 Description of safety assessment parameters

Adverse event

Any harmful reaction occurring in a person taking part in a biomedical research project whether or not related to the research or to the substance on which the research is being carried out.

Adverse effect of an investigational medicinal product

Any harmful undesired reaction to an investigational medicinal product regardless of the dose administered.

Serious adverse effect or event

Any adverse effect or event which results in death, is life-threatening to the person taking part in the research, requires hospitalisation or prolonged hospitalisation, causes severe or sustained incapacity or handicap or is reflected by a congenital abnormality or malformation involving a medicinal product regardless of the dose administered.

New finding

Any new safety data which may lead to a reassessment of the benefit/risk balance of the research or of the investigational medicinal product or which may be sufficient to consider amendments in terms of administration of the investigational medicinal product in conducting the research.

8.2 Methods and schedule of assessments, method for recording and analysis in the different safety parameter groups:

a) Study committees

- **Steering Committee**

  This will be made up of the clinicians who initiated the project, the biostatistician responsible for the project, representatives of the sponsor and of the clinical research unit appointed for the research. It will define the general organisation and conduct of the research and will co-ordinate information. Initially, it will establish the methodology and decide actions to be taken in unplanned situations during the search. It will monitor the conduct of the research in particular in terms of tolerability and adverse events.

- **Independent Review Committee**

  An independent review committee (Safety and Data Review Board) will be set up by the sponsor in order to monitor local complications, the haemorrhagic transformation rate and the death rate in both groups. It will meet after 102 patients have been included and at the request of the sponsor or co-ordinating investigator.

b) Safety assessment parameters: schedule, recording methods and analysis in the different groups

Any adverse events will be recorded during the first 24 hours and then on the D1, D7 and 3-month visits.

Recording methods:

Adverse events will be recorded onto the case report forms on D1, D7 and at 3 months.

Analysis in the different groups:

The following analyses will be performed in the 2 groups:

- Local RIPerC lower limb complication rate: deep venous thrombosis, acute limb ischaemia, skin lesions or others.
- Haemorrhagic transformation rate in cases of IV thrombolysis.
- Early neurological deterioration rate defined as an increase in the NIHSS score of over 4 points between D0 and D1.

8.3 Procedures for recording and notifying adverse events

a) Non-serious adverse event

Any adverse event – which is non-serious according to the above definition – occurring during the research and following the research must be recorded in the case report form in the section dedicated to this purpose.

A single event must be reported per item. The event may be a symptom, diagnosis or result of a further investigation deemed to be significant or clinical or para-clinical information which describes the corresponding event as well as possible must be recorded.

b) Serious adverse event (SAE)

The investigators must notify the sponsor, Versailles Hospital Centre, **immediately** of serious adverse events as defined above.

The investigator will complete the serious adverse event form (in the research case report form) and send these to the DRCI by fax at +33 (0)1 39 23 97 73 within 24 hours (if possible after an immediate telephone call to +33 (0)1 39 23 97 85 if a patient dies or life is unexpectedly threatened). The investigator must also inform the CRA responsible for the research about the occurrence of the SAE and URCPO for incident accounting purposes.

For each serious adverse event, **the investigator must enter an opinion on the causality of the event with each investigational medicinal product or any other treatments.** It may not be

possible to obtain the information concerning the description and assessment of an adverse event within the requisite time for the initial declaration.

Clinical progress and the results of any clinical assessments and diagnostic and/or laboratory investigations and any other information allowing an appropriate analysis of the causality relationship will be recorded:

-either on the initial SAE declaration if these are immediately available,

-or later and as soon as possible, sending a new supplemented SAE declaration by fax (stating that this is a follow-up to a declared SAE and the follow-up number).

All declarations made by the investigator must **identify each subject participating in the research by a unique code number** allocated to each subject.

If the death of a subject taking part in the research is reported, **the investigator will provide all of the further information requested** (hospitalisation report, post mortem results, etc., to the sponsor).

Any new finding which occurs during the research or in the context of the research originating from information in the literature or ongoing research must be notified to the sponsor.

- Declaration of serious adverse events to the Health Authorities

This declaration will be made by the DRCI after assessment of the severity of the adverse event, the causal relationship with each investigational medicinal product and any other treatments and the unexpected nature of adverse effects.

All suspected serious unexpected adverse effects will be declared by the sponsor to the competent authority within the legal deadlines.

If a serious adverse effect due to one of the research treatments or the research itself occurs, the competent authorities, Ethics Committee and research investigators must be informed.

8.4 Details and duration of follow-up of people with adverse events

Any patient who develops an adverse effect must be followed up until the effect has resolved or stabilised.

a) Non-serious adverse event

If the event is not serious its outcome will be recorded on the corresponding page of the case report form in the section intended for this purpose. A non-serious adverse event will be defined as severe pain (VAS ≥ 5) during the RIPerC protocol. In these cases, the doctor will be informed immediately.

b) Serious adverse event

If the event is serious, an SAE follow-up will be sent to the DRCI

Serious adverse events will be defined in terms of:

- Neurological deterioration during the RIPerC (≥ 4 NIHSS points/admission)
- Symptomatic haemorrhagic transformation (≥ 4 NIHSS points) on imaging on D1
- Acute lower limb ischaemia
- Per-conditioning lower limb deep venous thrombosis
- Per-conditioning lower limb skin lesion or skin bed sore
- Chest pain
- Acute respiratory distress
- Hypotension, SBP < 80 mmHg
- Severe hypertension SBP, ≥ 230 mmHg
- Major change in blood pressure. Difference in BP measurements of over 30 mmHg before and after the RIPerC protocol.
- Prolongation of hospitalisation,
- Deaths

or new findings occurring during the research or in the context of the research originating from information in the literature or ongoing research. It should be noted that in all clinical studies the RIPerC protocol has not caused any serious side effects or change in BP or HR.

9. Statistics

9.1 Statistical analytical plan

The statistical analysis will be performed at the end of the follow-up period for all patients and after the database has been frozen.

The interim analysis will be performed once the number n1 defined by the Posch and Bauer protocol has been reached, i.e., 102 patients with the parameters recorded and after the interim freezing of the database for the primary outcome.

The main details of the statistical plan are described in the paragraph below.

All confidence intervals will be calculated at a two-tailed 5% probability threshold and the results of the statistical tests will be given with a two-tailed 5% statistical threshold.

The analysis will be performed at the Paris-Ouest Clinical Research Unit (Prof. Ph. Aegerter). Data will be analysed on SAS 9.3 software (Cary Inc, North Carolina, USA) and using R language (v3 R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/).

9.2 Description of patients

Quantitative data will be described by their indices of central position (mean, median) and dispersion (standard deviation, interquartile range and range) and qualitative and ordinal data will be described by distributions and frequencies (proportions with their 95% confidence intervals).

The Chi 2 test will be used to compare distributions of qualitative variables whereas analysis of variance will be used to compare distribution of continuous quantitative variables, ordinal variables (or quantitative variables which are not normally distributed even after Box-Cox transformation, in order to improve the system or stabilise variance) compared by a non-parametric Kruskal-Wallis test.

9.3 Primary outcome

The primary outcome is the absolute change in brain infarction volume between D0 and D1 which is deemed to be a continuous variable. This will be analysed by a comparison of means test between the two groups taking account of stratification factors – centre and thrombolysis – consistent with EMEA recommendations [http://www.emea.eu.int Points to consider on adjustment for baseline covariates. CPMP/EWP/2863/99].

Adjustment variables: age; sex, NIHSS and admission blood glucose in a multivariate model will be considered as a secondary analysis.

9.4 Interim analysis

An interim analysis will take place using the Posch and Bauer method. This will be used to determine conditional power depending on the variance found during the first stage and the level of significance of the test performed at the end of this first stage providing the information required alongside the description of the distribution of adverse effects which is necessary for the independent review committee to come to a decision on continuation of the study.

9.5 Secondary outcome

The secondary end point concerning change in brain infarction volume between D0 and D1 will be assessed relatively between the two groups.

The other secondary outcome will be analysed by a multivariate regression model belonging to the GLM family adapted for the continuous nature (such as the scores → linear regression), qualitative nature (ordinal scores such as the 3 month Rankin score between 0 and 1, or binary scores such as the Barthel score ≥ 95, NIHSS neurological deterioration NIHSS D1 – NIHSS D0 ≥ 4, haemorrhagic transformation, Re-canalisation → binary or ordinal logistics) or censured data (death or score at 3 months → Cox model) of response taking account of stratification criteria and pre-specified adjustment variables. The effect of thrombolysis will be investigated routinely in terms of any interaction.

In all situations the shapes of the effects of continuous explanatory variables will be investigated, (fractional or polynomial), and the main interactions (particularly patient characteristics, treatment and prior thrombolysis) will be investigated and tests for the suitability of models will be applied.

For the tolerability analysis the adverse events will be described by frequency and presented by treatment arm, type of event, causal relationship (certain, probable, possible, unrelated) and intensity.

9.6 Analysis population

The efficacy analysis including the primary outcome, will be performed on an intention to treat basis.

In the intention to treat (ITT) analysis, all patients randomised into their arm will be assessed whether or not they have received the whole conditioning protocol.

The tolerability analysis will be performed on a per protocol (PP) basis and will include patients who have received part of the conditioning protocol compared to the other patients.

9.7 Methods for taking account of missing, unused or invalid data points

Patient status those selected, assessed on an intention to treat (ITT), or per protocol (PP basis), those lost to follow-up or who drop out of the study and deviations from the protocol will be described and compared by arm and by centre. Classification of deviations as minor or major will be performed blind on the basis of a list which is pre-determined by the steering committee.

Patients who are lost to follow-up, die or leave the study will be censured on the date of the latest news. When a patient is censured the last known result value will be used. All missing or unvalidated data points will, after a blind counter expertise by the independent review committee be replaced by the last available data for the corresponding parameters for that patient (LOCF “last observation carried forward” procedure). If a large number of data points are missing, a sensitivity analysis using multiple imputation by chained equations (MICE) will be performed.

As the primary outcome involves MRI measurements on D0 and D1, subjects who have missing data points on D0 will be excluded from the primary analysis, whereas the values on D1 may be used for an imputation procedure.

All missing data will be detailed in the final report.

9.8 Management of amendments made to the initial analytical plan

This plan may be reviewed after taking account of any amendments to the protocol, being adapted for the development of unexpected adverse events during the conduct of the study, which impacts the data analysis. Any such revisions will be performed before the database is frozen. These amendments or additional analyses will be deemed to be *post-hoc* analyses and will be recorded as such in the analysis and in the publication. These changes in statistical methods decided in retrospect will be approved by the Steering Committee and justified in an amendment to the protocol and in the study report.

10. Rights to access source data and original documents

In terms of rights of access to data and original documents, people who have direct access pursuant to current legislation and regulations, particularly articles L.1121-3 and R.5121-13 of the French Code of Public Health (such as the investigators, people responsible for quality control, monitors, clinical research associates, auditors and anyone, any people requested to collaborate in these studies) will take all necessary precautions in terms of confidentiality of information relating to investigational medicinal products, studies and people taking part in these studies, particularly with respect to their identity and to the results obtained. Data collected by these people during quality controls or audits will then be anonymised.

11. Legal and ethical requirements

11.1 Laws and good clinical practice

The research will be governed by the sponsor's standard operating procedure. The sponsor is defined by the 2004-806 law of 9 August 2004.

The Versailles Hospital Centre is the sponsor of this research, pursuant to section 2 of article L.1121-1 of the French Code of Public Health and the Versailles Clinical Research and Innovation delegation (DRCI) will undertake the regulatory tasks.

The sponsor will submit the dossier for approval to the Ethics Committee (Ile de France EC No. 11, St-Germain-en-Laye) in accordance with article L.1123-6 of the French Code of Public Health.

The research sponsor will be the owner of the data and the data may not be used or past to a third party without prior agreement.

The Versailles Hospital Centre reserves the right to stop the research at any time for medical or administrative reasons, in which case the investigator will be notified.

The principal investigator and investigators taking part in the study approve the final version of the protocol. Any investigator acting as the signatory of the protocol and any co-investigator included on the list of participants undertakes to carry out this research in compliance with law No. 2004-806 of 9 August 2004 on public health policy and consistent with Good Clinical Practice and the declaration of Helsinki. Before the research begins each investigator will provide the research sponsor's representative with a copy of his dated and signed personal curriculum vitae including his/her registration number in the Ordre des Médecins and his/her ADELI number.

11.2 Monitoring procedure

According to the Ile de France DIRC classification, this research project is risk level C.

The CRAs who represent the sponsor will carry out visits to the investigating centres at the frequency consistent with the patient follow-up plan in the protocol, with the inclusions in the different centres and with the level of risk attributed to the research.

- Opening visit for each centre: before inclusion, to set up the study and make the different people working in the biomedical research aware of the study.

- During subsequent visits, the case report forms will be reviewed as the research progresses by the CRA. The principal investigator for each centre and the other investigators who include patients or follow up people taking part in the research undertake to meet the CRA at regular intervals.

During these onsite visits and consistent with Good Clinical Practice, the following information will be reviewed:

-Compliance with the protocol and with the procedures defined for the research.

-Confirmation of patients informed consents

-Examination of original documents and a comparison with the data recorded in the case report form in terms of accuracy, missing data points and consistency of the data following the requirements drawn up in the DRCI procedures.

- Closure visit: recovery of case report forms, biomedical research documents and archiving.

11.3 Case report form: CRF

The case report form for visits D0, D1, D7 and M3 will include for each patient: the patient code made up of the centre code and a sequential patient inclusion number in the centre:

- The first letter of the subject's surname
- The first letter of the subject's forename
- The subject's date of birth
- The subject's date of inclusion
- The inclusion and non-inclusion criteria
- Clinical and biological variables of interest
- A clinical adverse event form.

The research data will be recorded using the Case Report Form drawn up with the assistance of the EMIC and monitored by a DRCI CRA. The modules corresponding to inclusion and to the D1 and D7 visits will be completed promptly in order to facilitate freezing the database and the interim analysis.

All of the information required by the protocol must be provided in the case report form and an explanation given by the investigator for each missing data point.

The data must be transferred into the case report form as they are obtained. This applies to both clinical and paraclinical data. Data relating to serious adverse events will be recorded on the SAE declaration form which will be faxed to the DRCI as soon as possible as required by law.

Incorrect data found in the case report forms will be replaced in the form by a declared investigator. The research CRA and Data Manager will also be able to view the CRF and

ask questions (queries) remotely.

Subjects' anonymity will be ensured by entering no more than the patient code in the research, the first letter of the subject's surname and the first letter of the subject's forename on all documents required for the research or by using appropriate measures to redact information (correction fluid, etc.) concerning named data on copies of original documents intended for the research documentation.

The data will be registered in an electronic database as they are communicated to the co-ordinating centre. A meeting bringing together all people working in the study will take place before the database is frozen in order to come to a decision on any requests for unresolved corrections, review the status of the database and for data quality control purposes. At this point no changes may be made to the data without unfreezing the database. After the database is frozen the data will be exported to the biometrics centre.

An application will be made to CNIL with respect to the electronic data file using the appropriate procedure for the situation before it is used.

11.4 Document archiving

The research documents governed by the biomedical research law must be archived by all parties concerned for a period of 15 years after the end of the research.

The following documents will be archived in the Versailles Hospital Centre DRCI premises:

-Copies of the ANSM authorisation letter and the mandatory EC opinion

-The successive versions of the protocol (identified by version no. and date of version),

-Correspondence with the sponsor,

-Subjects' signed consents in a sealed envelope with the corresponding inclusion list or register,

-The completed validated case report form for each subject included,

-All annexes specific to the study,

-The final study report from the statistical analysis and study quality control (a copy of which will be sent to the sponsor),

-Certificates of any audits conducted during the research

The person responsible for the analysis (on a paper or electronic support) must also archive the database from which the statistical analysis is performed.

11.5 Request from approval from ANSM

In order to be able to begin the research, the Versailles Hospital Centre, as sponsor, must submit an authorisation application dossier to the competent authority, ANSM (French National Health and Medicines Agency). The competent authority defined in article L. 1123-12, will issue a decision with regard to the safety of people taking part in a biomedical research project taking account in particular of the safety and quality of the products used in the research, in compliance where applicable with current reference standards, their conditions of use and the safety of the people concerned in terms of the procedures performed and methods used and the intended methods for following up these people.

11.6 Ethic committee

Consistent with article L.1123-6 of the French Code of Public Health, the research protocol must be submitted by the sponsor to an Ethics Committee. The opinion of this committee will be communicated to the competent authority by the sponsor before the research begins. It will also appear in the information leaflet given to patients.

11.7 Study amendments

The DRCI must be informed of any planned amendment to the protocol by the co-ordinating investigator. Amendments must be defined as substantial or non-substantial.

A substantial amendment is an amendment liable in one way or other to change the guarantees provided to people taking part in the biomedical research (a change to inclusion criteria, growth of inclusion period or participation of new centres, etc.).

After the research has begun, any substantial amendment to it made at the initiative of the sponsor must obtain approval from the committee and authorisation from the competent authority prior to being implemented. In this situation, if necessary, the committee will ensure that a new consent for people taking part in the research is correctly obtained.

In addition, any extension to the research (major amendment to the treatment regimen or populations included, extension of treatments or therapeutic procedures not intended initially in the protocol) will be deemed to be new research.

An application for authorisation must be made **by the sponsor** for any substantial amendment **following payment of a tax** and/or a request made for an opinion from the EC.

11.8 Information and consent letter

An information letter will be given and signed consent will be obtained for this study. In view of the urgent nature of acute brain infarction (<H6), the information and consent will be recorded using the details stipulated in article L 1122-1-2 of the 9 August 2004 law on biomedical research to be implemented in emergency situations.

If it is not possible to obtain prior consent from a patient because of his/her neurological state, the patient's consent will not be sought but, rather, consent will be sought from a member of the patient's family or family circle. The person concerned will be informed as soon as possible and ask for his/her consent for any continuation of the research.

In order to inform the patient and the patient's family circle as well as possible in this urgent situation, a consent form will be given to and signed by the people concerned: an information letter summarising the nature of the study together with its objectives and main risks + a document describing the methods, restrictions and risks of the study. This will describe the conditions under which patients participate and will state that the participants may leave the study at any time without providing an explanation. The participants are allowed to freely question the investigators about the protocol before signing the consent. This document must include the version number and date of the version presented to the EC followed by the date and version number agreed by the EC (in the event of an amendment, the numbering should be continued for successive versions).

11.9 Declaration to CNIL

Legislation required that the declaration of an electronic file containing personal data collected for the research must be made before the research actually begins.

A **specific reference methodology was produced by CNIL in January 2006 for the processing of personal data in biomedical research defined by the 2004-806 Law of 9 August 2004.** This will apply, as the study falls within the scope of articles L.1121-1 and subsequent articles of the French Code of Public Health. This methodology enables a **simplified declaration procedure** to be used when the nature of data collected in the research is compatible with the list laid down by CNIL in its reference document.

When the protocol involves data quality control by a CRA representing the sponsor and falls within the scope of the CNIL simplified procedure, the DRCI as sponsor will ask the person responsible for the electronic file to undertake in writing to comply with the simplified reference methodology MR06001.

11.10 Final report

The final research report will be written in collaboration with the research coordinator and by a statistician. This report will be submitted to each of the investigators for their opinion. Once a consensus has been obtained the final version will be validated by the signature of each of the investigators and sent as soon as possible to the sponsor after the actual end of the research. A report written using the reference plan from the competent authority must be sent to the competent authority and to an EC within a period of one year after the end of the research which is defined as the last follow-up visit of the last subject included. This period is shortened to 90 days if the research is stopped early.

12. Insurance and scientific responsibility

12.1 Insurance

The Versailles Hospital Centre is the sponsor for this research. Consistent with the law on biomedical research it has taken out insurance with S.H.A.M (Société Hospitalière d'Assurances Mutuelles, SHAM, Lyon) for the entire duration of the research which guarantees its own civil liability and that of any person involved (doctor or staff involved in conducting a research) (law no. 2004-806, Art L.1121-10 of the French Code of Public Health).

12.2 Scientific undertaking

Each investigator will undertake to meet the legal requirements and conduct the research according to GCP respecting the terms of the current declaration of Helsinki. In order to do this, a copy of the **scientific undertaking (Versailles Hospital Centre DRCI standard document)** which is dated and signed **by each investigator** from each clinical department in a participating centre, will be given to the sponsor's representative.

13. Publication rules

The Versailles Hospital Centre is the owner of the data. They may not be used by a third party without its prior agreement.

The leading signatories of publications will be those people who have actually taken part in constructing and carrying out the protocol and the analysis and writing of the results according to international publication rules. The manuscript will follow the CONSORT group guidelines for presenting results of a randomised study in patients.[57]

The Versailles Hospital Centre must be mentioned as the sponsor of the biomedical research and as the funding support where applicable.

The following details must appear on the manuscripts or either oral or poster presentation supports:

- the Versailles Hospital Centre as the sponsor of the biomedical research,
- the contribution of the partners who funded all or part of the research,
- the exact affiliation titles of each of the authors must meet the affiliation-naming requirements for each of the bodies to which members of the scientific committee belong and those of the investigators from the different participating centres in order to enable publications to be correctly referenced in the SIGAPS system.
- the publication will also mention support from the Paris-Ouest URC.

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15. Annexes

Annex 1: National Institute of Health Stroke Scale (NIHSS). Stroke severity score

Instructions	Score	Actual score
1a **Level of consciousness (0-3)** Verbal or painful stimulation of the patient (left to the choice of the examiner)	**0** Aware, responds briskly. **1** Responds to minor stimuli **2** Responds to repeated or painful stimuli **3** Stereotypic or flaccid response	
1b **Consciousness level (0-2)** - "How old are you?" - "What month is this?"	**0** Correct answer to the 2 questions **1** One single correct answer or **intubated**, or severe dysarthria or language barrier **2** No correct response or **aphasic**	
1c **Consciousness-commands (0-2)** - "Open and close your eyes" - "Close and open your hand" *To be imitated if the command is not followed*	**0** Executed the 2 commands correctly **1** One correct response **2** No response	
2. Horizontal oculomotilities (0-2) Test voluntary and reflex horizontal movements without a heat test (finger following): "Follow my finger"	**0** Normal oculomotility **1** Conjugate deviation of the eyes which may be reduced by voluntary or reflex activity or isolated damage to a cranial nerve **2** Complete paralysis of the side	
3. Visual field (0-3) Test the visual field by quadrants (upper and lower) using finger counting or if necessary the menace blink reflex.	**0** Normal visual field **1** Partial HLH or loss of vision **2** Complete HLH **3** Dual HLH or cortical blindness	
4. Facial paralysis (0-3) "Show me your teeth, raise your eyebrows and close your eyes" *To be imitated if command not performed or Pierre Marie and Foix manoeuvre*	**0** Normal **1** Slight central facial paralysis (FP) **2** Clear central PF (total inferior) **3** Dual PF or total PF	
5. Upper limb motility 5.1 "Stretch out your arm and left hand" **(0-4)** 5.2 "Stretch out your arm and right hand" **(0-4)** for 10 seconds	**0 N**ormal **1** Resists a weight (arm falls within 10 sec) **2** Does not resist (arm touches the bed before 10 sec). **3** Does not lift limb (contraction without movement) **4** No movement	–
6. Lower limb motility 6.1 "Stretch out your left leg" (30°) **(0-4)** 6.2 "Stretch out your right leg" **(0-4)** for 5 seconds	**0** Normal **1** Resists a weight (arm falls within 5 sec) **2** Does not resist (arm touches the bed before 5 sec) **3** Does not lift limb (contraction without movement) **4** No movement	- - - - - - -

7. Limb ataxia (0-2) "Place your index finger on your nose" "Place your heel on the opposite knee" *Bilateral manoeuvre*	**0** Normal or impossible because of paralysis or aphasia **1** Ataxia of one limb **2** Ataxia of 2 limbs ***9*** *Amputation or joint block*	
8. Sensitivity (0-2) Examine sensation to pin prick or withdrawal following nociceptive stimulation if confused or aphasic (arm, leg, face, trunk, bilaterally)	**0** Normal. **1** Hypoesthesia or aphasia or stupor **2** Severe to total deficit	
9. Language (0-3) "Describe the following scene" (*cf.* fig.1 p 3) "Tell me the name of these objects" (*cf.* fig.2) "Read these phrases" (*cf.* text 1 p 4) If visual disturbance, identify the objects in a hand and have the words repeated *Assess writing in an intubated patient*	**0** Normal **1** Aphasic but communicates **2** Quasi-impossible communication **3** Total aphasia, mutism or **coma**	
10. Dysarthria (0-2) "Repeat the following words" (*cf.* text 2 p 3) ***including*** *in aphasic patients*	**0** Normal articulation **1** Comprehensible **2** Incomprehensible, anarthria or mutism ***9*** *Intubation or mechanical obstruction*	
11. Extinction or negligence Test bilateral simultaneous sensitivity Test perception in both temporal visual fields simultaneously Investigate for anosognosia and visuospatial neglect	**0** No extinction or complete HLH (if sensory extinction) and vice versa or aphasia or gives the impression of understanding **1** Extinction of one modality **2** Extinction of several modalities or visuospatial neglect or anosognosia	
	TOTAL SCORE	

Annex 2. Modified Rankin score, mRS

0: No symptoms

1: Symptoms without any incapacity (able to perform all usual activities)

2: Mild incapacity (unable to perform all usual activities but able to look after his/her affairs alone)

3: Moderate incapacity (requires assistance but walks alone)

4: Severe incapacity (requires assistance for walking and physical body needs)

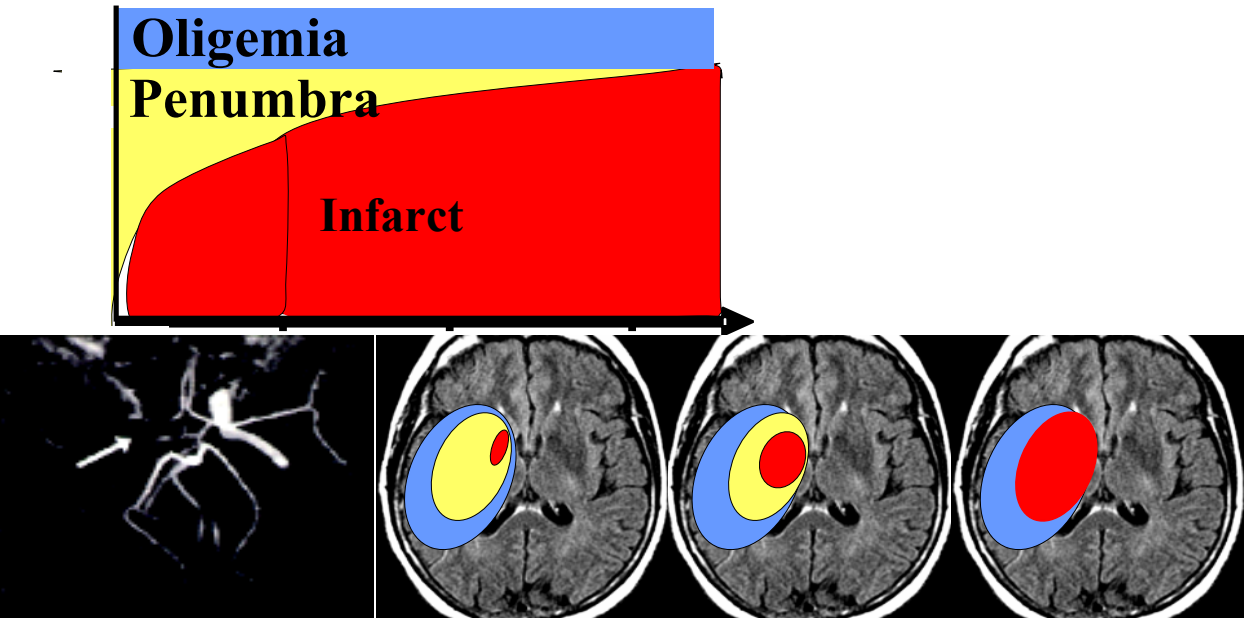
5: Severe incapacity (bedbound, incontinent, permanent surveillance required)

6: Dead

Annex 3. Barthel Score

Item	*Description*	*Score*	*Dates*	
1.Alimentation	Autonome. Capable de se servir des instruments nécessaires. Prend ses repas en un temps raisonnable A besoin d'aide, par exemple pour couper	10 5		
2.Bain	Possible sans aide	5		
3.Continence rectale	Aucun accident Accidents occasionnels	10 5		
4.Continence urinaire	Aucun accident Accidents occasionnels	10 5		
5.Déplacements	N'a pas besoin de fauteuil roulant. Autonome sur une distance de 50 m, éventuellement avec des cannes. Peut faire 50 mètres avec aide. Autonome dans un fauteuil roulant, si incapable de marcher.	15 10 5		
6.Escaliers	Autonome. Peut se servir de cannes. A besoin d'aide et de surveillance.	10 5		
7.Habillement	Autonome. Attache ses chaussures. Attache ses boutons. Met ses bretelles. A besoin d'aide, mais fait au moins la moitié de la tâche dans un temps raisonnable.	10 5		
8.Soins personnels	Se lave le visage, se coiffe, se brosse les dents, se rase. Peut brancher un rasoir électrique.	5		
9.Usage des WC	Autonome. Se sert seul du papier hygiénique, de la chasse d'eau. A besoin d'aide pour l'équilibre, pour ajuster ses vêtements et se servir du papier hygiénique.	10 5		
10.Transfert du lit au fauteuil	Autonome, y compris pour faire fonctionner un fauteuil roulant. Surveillance ou aide minime. Capable de s'asseoir, mais a besoin d'une aide maximum pour le transfert.	15 10 5		

Figure 1: Penumbra time profile



MRA : Right cerebral middle artery occlusion occlusion (Arrow)

Figure 3. Biological effects of remote ischaemic conditioning. *Taken from Kharbanda Lancet 2009* [26]

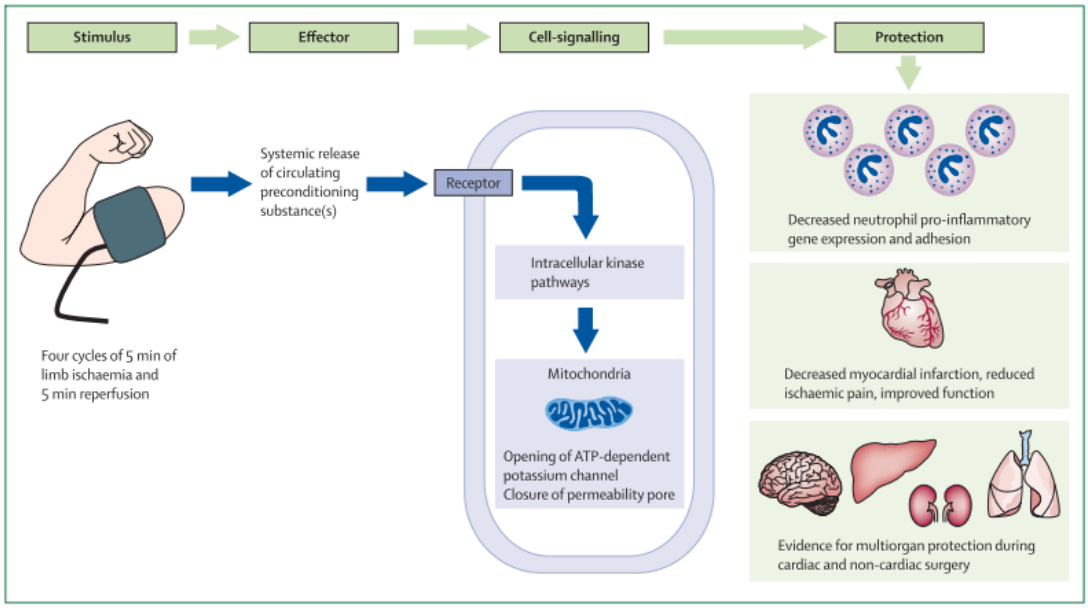
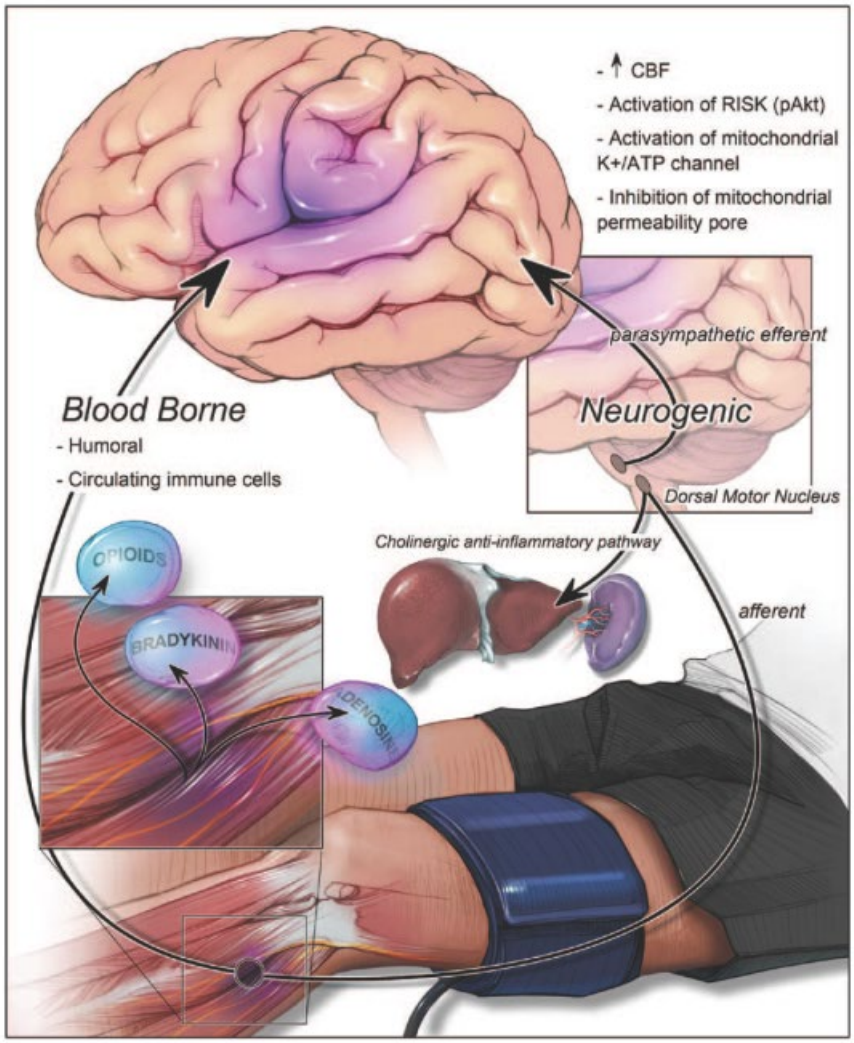


Figure: **Biological effects of remote ischaemic preconditioning**
Transient ischaemia of the arm liberates a circulating effector that induces remote cellular adaptation to a subsequent, extended, and potentially lethal period of ischaemia in remote tissues.

Figure 4. Supposed or demonstrated neuroprotection mechanisms during Remote ischaemic preconditioning.
Taken from Hess et al. Stroke 2013 [13]


- ↑ CBF
- Activation of RISK (pAkt)
- Activation of mitochondrial K+/ATP channel
- Inhibition of mitochondrial permeability pore
parasympathetic efferent
Neurogenic
Dorsal Motor Nucleus
Blood Borne
- Humoral
- Circulating immune cells
Cholinergic anti-inflammatory pathway
afferent
OPIOIDS
BRADYKININ
ADENOSINE

CV of co-ordinating investigator

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- Clinical research studies: DETECT (n= 11) , ARCH (n=8), PERFORM (n=6), FIND (n=14), CLOSE (n=0), INTERACT (n=8)
- Publications:
  - Legriel S, Schraub O, Azoulay E, Hantson P, Magalhaes E, Coquet I, Bretonniere C, Gilhodes O, Anguel N, Megarbane B, Benayoun L, Schnell D, Plantefeve G, Charpentier J, Argaud L, Mourvillier B, Galbois A, Chalumeau-Lemoine L, Rivoal M, Durand F, Geffroy A, Simon M, Stoclin A, Pallot JL, Arbelot C, Nyunga M, Lesieur O, Troché G, Bruneel F, Cordoliani YS, Bedos JP, Pico F. Critically III Posterior Reversible Encephalopathy Syndrome Study Group (CYPRESS). Determinants of recovery from severe posterior reversible encephalopathy syndrome. **PLoS One. 2012;7(9):e44534. (IF=4)**
  - Pico F, Jacob MP, Labreuche J, Soufir N, Touboul PJ, Benessiano J, Cambien F, Grandchamp B, Michel JB. Amarenco P. Matrix metalloproteinase-3 and intracranial arterial dolichoectasia: MMP-3 and Intracranial Arterial Dolichoectasia. **Ann Neurol. 2010;67:508-515(IF=10)**
  - Pico F, Labreuche J, Seilhean D, Duckyaerts C, Hauw J-J, Amarenco P. Association of small vessel disease with dilatative arteriopathy of the brain: neuropathological evidence. **Stroke. 2007;38: 1197-1202. (IF=5.7)**
  - Pico F, Labreuche J, Touboul PJ, Leys D, Amarenco P. Intracranial arterial dolichoectasia and small-vessel disease in stroke patients. **Ann Neurol. 2005 Apr;57(4):472-9. (IF=7)**
  - Pico F, Labreuche J, Cohen A, Touboul PJ, Amarenco P. Intracranial arterial dolichoectasia is associated with enlarged descending thoracic aorta. **Neurology 2004 63(11):2016-21. (IF=5)**

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SIGNATURE:
(Signed)