Protocol

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Endovascular treatment compared to intravenous thrombolysis for acute ischemic stroke

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This supplement contains the following items:

1. Original protocol (“*Version 1 – August 2007”),* final protocol (“*Version 2 – December 2008”),* summary of changes.

2. Statistical analysis plan, embedded in the two protocol’s versions.

SYNTHESIS Expansion

A randomized controlled trial between loco-regional intra-arterial (IA) and systemic intravenous (IV) thrombolysis with Alteplase in acute ischemic stroke

Prot. SYNTHESIS Expansion_07 – Version 1 – August 2007

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1. SYNOPSIS

Title	A randomized controlled trial between loco-regional intra-arterial (IA) and systemic intravenous (IV) thrombolysis with Alteplase in acute ischemic stroke.
Study objectives	**Primary:** assess whether IA thrombolysis, as compared to the administration of Alteplase IV, increases survival free of disability (modified Rankin score of 0 or 1) at 3 months. **Secondary:** assess in the two treatment groups: • the neurological deficit 7 days after thrombolysis; • the safety of the procedure on the basis of events reported within 7 days following thrombolysis: symptomatic cerebral hemorrhage, fatal and non-fatal stroke, death from any cause, neurological deterioration.
Study design	Randomized, controlled, multicenter, open-label clinical trial with blinded follow-up. Phase III Study.
Study population	Patients with acute, symptomatic ischemic stroke, shown on CT, with the following conditions are eligible : 1. possibility of starting IV Alteplase treatment within 3 hours from stroke onset; 2. possibility of starting IA thrombolysis within 6 hours from stroke onset; 3. uncertainty of the most appropriate choice.
Inclusion criteria	• Sudden focal neurological deficit attributable to a cerebral stroke • Clearly defined time of onset, allowing initiation of IV treatment within 3 hours and IA treatment within 6 hours of symptoms onset. • Age between 18 and 80 years. • Availability of an interventional neuroradiologist.
Exclusion criteria	The exclusion criteria coincide exactly with those found in the Alteplase technical form *(see protocol for details)*. To those the following have been added: • Known contrast sensitivity; • Women of childbearing potential or known to be breastfeeding; • Prognosis very poor regardless of therapy (likely to be dead within months); • Disability preceding stroke (i.e. modified Rankin scale of >1); • Unlikely to be available for follow-up (i.e. no fixed home address, visitor from overseas); • Refused consent; • Any other condition which the investigator feels could pose a hazard in terms of risk/benefit to the patient, or if the therapy proves impracticable.
Computed tomographic (CT) scan exclusion criteria	• Intracranial tumors, except small meningioma. • Hemorrhage of any degree. • Acute infarction (since this may be an indicator that the time of symptoms onset is not correct).
Treatment plans	**Experimental arm:** IA thrombolysis with Alteplase up to 0.9 mg/Kg (max 90 mg), administered with microcatheters, introduced preferably at the femoral artery, and/or with mechanical devices (fragmentation/retraction/aspiration). The neuroradiologist may choose the procedure most appropriate to the circumstances and administer a lower dose of IA Alteplase than the maximum allowed, in case of re-canalization of the vessel. The IA procedure must start as soon as possible and no later than 6 hours from stroke onset.

	Control arm: Alteplase in a dose of 0.9 mg/kg of body weight (maximum 90 mg), administered as intravenous infusion over 60 minutes, 10% of which is given as an initial intravenous bolus.
Calculation of sample size	The estimation of sample size for the primary *outcome* is based on a standard test of two samples for differences in binomial proportions (two-tailed test) with alpha = 5% and power = 80%. The study seeks to verify or refute an absolute difference of about 15% in the percentage of patients with a favorable *outcome* between the two treatment groups. At least 172 patients per arm should be enrolled, assuming that 40% of patients treated with IV Alteplase (estimate is based on patients treated with Alteplase in other studies) should provide a favorable *outcome*.
Statistical analysis	"Intention to treat" analyses will be used throughout the study. The analyses will be made by a statistician, blinded with respect to treatment which will be coded "A" or "B". The protocol will have two separate analyses: primary analysis and secondary analysis. Primary analysis will assess the effect of IA thrombolyis compared to IV Alteplase on survival and autonomy after 90 days. Those patients with modified Rankin scores of 0 or 1 are considered self-sufficient, the others are disabled or dead (2, 3, 4, 5 or 6 on the modified Rankin scale). Statistical analysis will be carried out using a 0 to 1 track score of the Rankin scale noted above as the *endpoint* of the study. This score will be tabulated by type of treatment (IA or IV). The result of the cross-tabulation will be assessed with a two tailed exact Fisher test, in parallel with calculation of the Mantel-Haenszel odds ratio ψ and the confidence interval ψ95%. Secondary analysis includes the following sub-analysis: a) analysis of the proportion of patients reaching an NIHSS score of ≤ 6 at day seven after thrombolysis, which will be conducted with Fisher's exact test; 2) assessment of the number of symptomatic intracranial hemorrhages, fatal and non-fatal strokes, death from any cause, neurological deterioration, in the two treatment groups, which will be compared with Fisher's exact test and binomial test. Subgroups analyses will then be conducted according to main baseline prognostic variables (age, severity of neurological deficit, time to randomization from stroke onset, CT scan, arterial fibrillation, diabetes mellitus, hypertension and the causes of stroke) *(see protocol)*.
Safety and interim analysis	During the period of recruitment two interim analyses are planned: the first after the first 100 patients randomized and the second after the second 100 patients (which means after 200 patients randomized). The Safety and Monitoring Committee will suspend the study if a statistical and a clinical imbalance in the risk to benefit ratio is found.
Study duration	Beginning recruitment date : February 2008 End recruitment date: September 2011.

2. RATIONALE

Stroke is a major public health concern in Italy, where its incidence is approximately 155.000 new cases (and 39.000 recurrences) per year. It represents the third cause of death after cardiovascular and neoplastic diseases, and is the cause of 10-12% of all deaths/year. Acute (30 days) mortality for stroke has been evaluated to be equal to 20% of all cases in Italy, while during the first year it is quantifiable as 30%. One year after stroke, one third of the surviving subjects show an elevated degree of disability, sufficient to define them as totally dependent (1).

There is evidence that IV thrombolysis is the only effective treatment to work in ischemic stroke within the first few hours of symptoms onset so far. Risks and benefits of thrombolysis are summarized in the systematic review of randomized controlled trials (RCTs) produced by The Cochrane Collaboration: thanks to thrombolytic therapy with IV Altaplase, which is approved for the treatment of ischemic stroke within three hours from onset in several countries, 103 more patients were alive and independent at the end of follow up for every 1000 patients treated (2).

For over 25 years few interventional neuroradiologists have been successfully treating this category of patients by an IA route. However, evidence is still required to support the clinical feeling that IA treatment, which needs longer time and greater complexity, indeed leads to a better outcome with respect to the IV approach (3, 4).

- *Pros and cons of IA thrombolysis*

  IA thrombolysis might offer many advantages over the IV route: such as being able to titer the dosage of the thrombolytic agent, to ensure a high drug concentration locally and low concentration in the systemic circulation, to facilitate recanalization with mechanical thrombolysis, to extend the therapeutic time window. However, compared to IV thrombolysis, the IA strategy requires more advanced technology and human resources and is consequently limited to highly specialized centers. Moreover, IA thrombolysis not only implies an expertise in neuro-intervention but also an organization that requires the prompt availability of a neuro-interventionist, a stroke team and a consolidated fast track to the angiography room.

- *Proof of effectiveness*

  The Cochrane review (2) identified only two RCTs on IA thrombolysis for acute ischemic stroke: PROACT(5) and PROACT II (6). Both studies compared the use of recombinant pro-urokinase (r-pro-UK) plus IA heparin or IV heparin only in patients with middle cerebral artery occlusion, randomized within 6 hours from symptoms onset. The meta-analysis of the two trials shows that IA treatment increases the risk of symptomatic intracranial hemorrhage (absolute risk increment 7% - a similar increase is observed with IV Alteplase,) but reduces the percentage of patients dead or dependent at long-term (absolute risk reduction 13%). However, this confidence interval was wide, due to the small sample size, and included the possibility that IA treatment might prevent even 1 dead or dependent patient per 100 treated. After the last update of the Cochrane review, another two RCTs were published on IA thrombolysis. In a first study (7), 16 patients with stroke and angiographic evidence of posterior circulation occlusion were randomized to be treated within 24 hours after stroke onset with IA UK or no treatment (control). All patients were acutely anti-coagulated. Four of eight patients who received IA UK compared with seven of the eight in the control group were dead or disabled at six months and there were four deaths in each treatment group. This small RCT, which was stopped before reaching the 200 patients planned on, due to slow recruitment and withdrawal of UK from the market, was definitely underpowered, and the small difference in outcome between the two groups may be explained by the play of chance. The second study (8), compared IV UK with IA UK administered within 6 hours of symptoms onset, was also stopped early because of 7 deaths: 4 in the group of 14 with IV treatment and 3 in the group of 13 treated with IA. Although the patients treated with IA in this study saw early improvement and to a higher degree, there was no difference in primary and secondary *outcomes* between the two groups.

- *Conclusion*
  Evidence on acute stroke management with IA thrombolysis is still scarce; there is only one RCT which compares the two approaches (IA and IV), stopped early. Moreover, previous RCTs were aimed to assess the efficacy of a specific thrombolytic drug rather than the complete IA approach. Indeed, patients were randomized after angiography (i.e. angiography and its associated risks were not considered as an integral part of the IA approach) and the IA procedure was strictly standardized (for instance mechanical devices were forbidden).
- *Innovation of Synthesis compared to previous studies*
  Synthesis is a pragmatic multicentric RCT that takes into account the above mentioned issues in order to compare the IA and IV in clinical practice:
  1. inclusion and exclusion criteria are based on those used for IV treatment with Alteplase;
  2. patients are considered for the study when uncertainty about appropriateness of the two approaches exists;
  3. patients are randomized before angiography, which is considered an integral part of the IA approach;
  4. patients randomized to IV Alteplase are treated within 3 hours from stroke onset;
  5. patients randomized to IA thrombolysis are to be treated as soon as possible but can be treated also after 3 hours from symptoms onset (but never more than 6 hours) as the time taken for IA approach is considered an integral part of treatment;
  6. IA thrombolysis can be both pharmacological and mechanical (the procedural choices of the interventional neuroradiologist depend on the type of occlusion, circumstances and personal experience);
  7. the sample size was planned considering a consistent superiority of IA over IV thrombolysis, to justify the use of a complex procedure that requires an increase in the amount of resources.
- *Possible impact of IA thrombolysis in clinical practice*
  The number of centers able to perform IA thrombolysis is definitely inadequate compared to the burden of patients with stroke but is probably sufficient to collaborate in a multicenter trial to clarify the role of IA thrombolysis for acute stroke management. The effort is worthy of a trial because a positive result could justify an allocation of resources in this direction and more widespread use of this treatment. Indeed, if IA thrombolysis proves so effective compared to IV thrombolysis then it should become available for most people, not just for the few lucky enough to be admitted to a specialist tertiary referral center.

3. START-UP/FEASIBILITY PHASE AND EXPANSION PHASE

The present expansion phase of the *SYNTHESIS* study, called *SYNTHESIS Expansion*, follows the *SYNTHESIS* study, which began in January 2004 is still on-going and will be concluded with the start of the present protocol. The *SYNTHESIS* study, which involved the Department of Neurosciences of the Niguarda Ca' Granda Hospital as coordinating center and 3 other centers in the Lombard region (*Spedali Civili* in Brescia, *S. Raffaele* Hospital in Milan, *Valduce* Hospital in Como), demonstrated the feasibility of the study. The feasibility phase was monitored by the Safety and Monitoring Committee composed of Prof. Livia Candelise, University of Milan, Prof. Peter Sanderkock, University of Edinburgh, and Prof. Gregory del Zoppo, University of Washington in Seattle.

The present expansion phase of the study intends to involve at least 10-20 centers with experience in endovascular interventions and equipped with a Stroke Unit, able to recruit 350 patients over a two year period. The *SYNTHESIS Expansion* protocol has been modified with respect to the earlier *SYNTHESIS*. The most relevant modification consists in having expanded the possibilities of endovascular interventions, leaving ample discretion in the choice of the approach to use. Therefore, the study is no longer a comparison between IV Alteplase and IA Alteplase but rather between IV Alteplase and endovascular intervention. In short, the crucial question to be answered by the study is: if it is better to trust a patient to the interventional neuroradiologist rather than treat him/her with

IV Alteplase, according to a pragmatic approach which reflects what actually happens and the modifications of recent years in the field of endovascular intervention. The interventional neuroradiologist is therefore free to use any mechanical *device* to splinter, melt or extract the thrombus.

4. OBJECTIVES

4.1 Primary aims

To assess whether local IA thrombolysis, as compared to IV Alteplase, increases survival free of disability (modified Rankin score of zero or 1) at 3 months.

4.2 Secondary aims

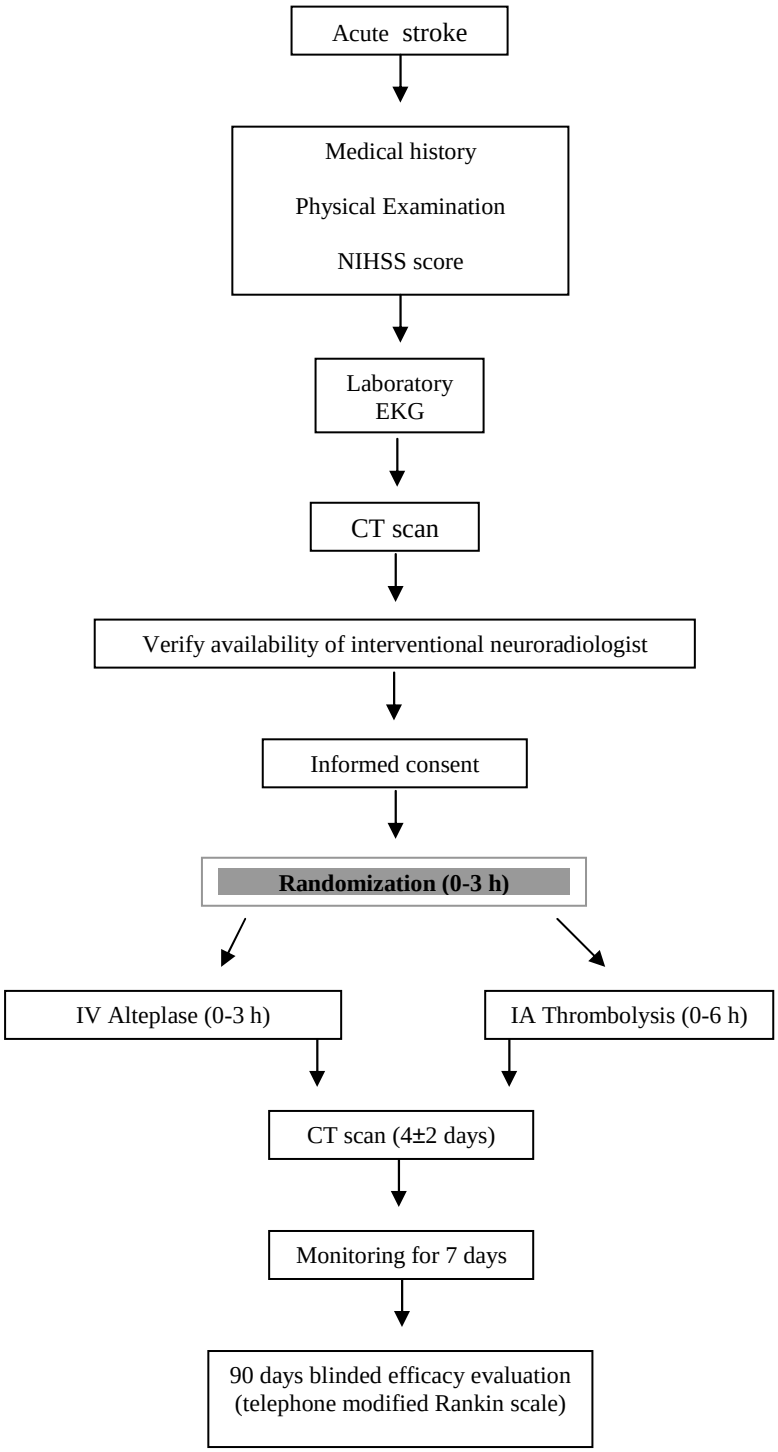
To assess in the two treatment groups:

1. the neurological deficit 7 day after thrombolysis;
2. the safety of the procedure on the basis of events reported within 7 days after thrombolysis: symptomatic intracranial hemorrhages, fatal and non-fatal stroke, death from any cause, neurological deterioration.

5. STUDY DESIGN

SYNTHESIS Expansion is a randomized, controlled, multicenter, open-label clinical trial with blinded follow-up, that proposes to verify if IA thrombolysis, as compared to IV thrombolysis with Alteplase, within 3 hours from ischemic stroke onset, increases the number of autonomous patients at 90 days.

5.1 Study outline - FLOW CHART



6. PATIENT ELIGIBILITY

Patients with acute, symptomatic ischemic stroke, shown on CT, with the following conditions are eligible :

1. possibility of starting IV Alteplase treatment within 3 hours from stroke onset;
2. possibility of starting IA thrombolysis within 6 hours from stroke onset;
3. uncertainty of the most appropriate choice.

6.1 Clinical inclusion criteria

- Sudden focal neurological deficit attributable to a cerebral stroke.
- Clearly defined time of onset, allowing initiation of IV treatment within 3 hours and IA treatment within 6 hours of symptoms onset.
- Age between 18 and 80 years.
- Availability of an interventional neuroradiologist.

6.2 Clinical and laboratory exclusion criteria

- Severe stroke as assessed clinically (e.g. NIHSS>25) and/or adequate imaging techniques
- Rapidly improving minor neurological deficit
- Clinical presentation suggestive of a subarachnoid hemorrhage (even if CT scan is negative)
- Seizure at onset of stroke
- Coma at onset
- Prior stroke within the last 3 months
- Any history of prior stroke and concomitant diabetes mellitus
- Major surgery or significant trauma in past 3 month
- Recent or present acute or dangerous bleeding
- Known hemorrhagic diathesis
- Patients in treatment with oral anticoagulants
- Administration of heparin within the previous 48 hours and a PTT exceeding the normal higher limit for the laboratory
- Recent (<10 days) external heart massage, obstetrical delivery or puncture at a non compressible site (e.g. subclavian or jugular vein puncture)
- Previous history of or suspected intracranial hemorrhage
- Previous history of central nervous system damage (neoplasm, aneurysm, intracranial surgery)
- Documented ulcerative gastrointestinal disease in the last 3 months, esophageal varices
- Severe liver disease, including hepatic failure, cirrhosis, portal hypertension (esophageal varices) and active hepatitis
- Arterial aneurysm, vascular malformations
- Neoplasm with increased bleeding risk
- Bacterial endocarditis, pericarditis
- Acute pancreatitis
- Severe hypertension: PAS >185 mmHg or PAD >110 mm Hg uncontrolled or requiring continuous IV therapy
- Baseline blood glucose <50 mg per deciliter (2.75 mmol/L) or >400 mg per deciliter (22mmol/L)
- Platelet count < 100.000/mm^3
- Known contrast sensitivity
- Women of childbearing potential or known to be breastfeeding
- Prognosis very poor regardless of therapy (likely to be dead within months)
- Disability preceding stroke (e.g., modified Rankin scale >1)
- Unlikely to be available for follow-up (e.g., no fixed home address, visitor from overseas)
- Refuses consent

- Any other condition which investigators feel would pose a significant hazard in terms of risk/benefit to the patient, or if therapies are impracticable.

6.3 Computed tomographic (CT) scan exclusion criteria

- Intracranial tumors except small meningiomas
- Hemorrhage of any degree
- Acute infarction (this may be an incorrect indicator of time of onset)

6.4 Principles of treatment uncertainties and patient selection

The researcher must be uncertain as to the best treatment to administer (IA or IV) to be able to randomize. To make a therapeutic decision, the possibility of considering non invasive examinations to visualize the occlusion (such as MR angiography, CT angiography or Doppler US) or the compromised cerebral area (diffusion MRI studies and/or perfusion CT/MRI studies) is not excluded. However, as there are uncertainties as to the use of patient selection in thrombolytic therapies with the above mentioned methods, these exams have not been considered essential for this study. They can therefore be used at the discretion of the researcher provided their use is noted in the CRF before randomization (Appendix A).

6.5 NON RANDOMIZED ELIGIBLE PATIENTS

A register will be kept for non-randomized patients, which will include the patient's initials, sex, age, date of observation, and reason for exclusion. (CRF "Form for non-randomized patients eligible for treatment with thrombolysis within 3 hours" Appendix C). These patients will be not followed-up.

7. RANDOMIZATION

The study provides for a simple randomization that will be carried out *on line* in a centralized way after filling out, again *on line*, data from the "CRF before randomization" (Appendix A). Randomization will be performed by the neurologist or emergency doctor who visits the patient in the emergency room. It will be done with hardware and software techniques, using a computer with a GNU/Linux operating system, equipped with connections to a series of radio devices (www.random.org). These will be tuned to different frequencies (where there are no artificial signals), making it possible to pick-up white sounds generated by the atmosphere. The noise is made into a sample with 8 bit and 8khz signal algorithms, that are filtered and converted into sequence of binary digits to high entropy.

The numbers generated are tested according to the recommendations of the U.S. National Institute of Standards and Technology (NIST). In particular, before being assigned to a treatment or another (IA Alteplase or Alteplase IV), the sequence 0/1 of the bits of randomization will be tested with the calculation of the integral

$$\operatorname{erfc}\left(\frac{|S|}{\sqrt{2}}\right) = p = \frac{2}{S\sqrt{2}} \int_{x}^{t^3} e^{-t^2} dt$$

in wich $S = b_1 + b_2 + \ldots + b_n$ is the sum of the sequence of bits. The sequence will be accepted as be random if p <0.05.

8. STUDY TREATMENTS

The study provides a comparison of the two treatments:

- *Experimental treatment (IA thrombolysis):* Alteplase to 0.9 mg/kg (max 90 mg), administered by IA, by means of a microcatheter preferably introduced via femoral artery and/or mechanical thrombolysis (fragmentation/retraction/aspiration). The neuroradiologist has the possibility of

choosing the procedure that best suits the situation and of administering a dose of IA Alteplase less than the maximum allowed, in case of recanalization of the vessel. The IA procedure must start as rapidly as possible and no later than 6 hours from stroke onset.

- *Standard treatment (IV thrombolysis):* the recommended dose is 0.9 mg of Alteplase/kg body weight (max 90 mg) administered as an intravenous infusion, 10% of which is given as a bolus followed by delivery of the remaining 90% as a constant infusion over 60 minutes.

8.1 Description of study procedures

8.1.1 IA Thrombolysis

- *Premise*

  IA procedure can vary according to circumstances, type of occlusion and experience of the health professional. While taking this into account, it is necessary to establish a certain homogeneity in IA treatment between the different participating units, both through the indications that follow and the continuous exchange of information and experience among researchers in the study. Variations in procedure are allowed as long as they are recorded in the in-hospital evaluation form.
- *Timing*

  The IA procedure must be performed as soon as possible after randomization and, in any case, within 6 hours from symptoms onset (taking into account possible impediments such as difficulty of catheterization or the need for anesthetics).
- *Anesthetic assistance*

  It is recommended that the availability of anesthetic assistance be evaluated at randomization. The need for anesthetic sedation in order to carry out a procedure is discretionary and must be reported in the CRF after randomization (Appendix B).
- *Cerebral arteriography*

  Arteriography precedes the therapeutic phase and must be targeted to acquiring data essential for making the endovascular therapeutic choices.

  Anticoagulant therapy, although recommended, is performed at the discretion of the health professional according to the current standard adopted in the single centers, it must be reported in the CRF after randomization (Appendix B). If anticoagulant therapy is used, an initial administration of 5000 IU of IV heparin in bolus is recommended, followed by 500IU/h infusion until the conclusion of the angiography.

  Once the diagnostic information has been acquired (site of occlusion, cerebral circulation, collaterals), the health professional can consider different therapeutic strategies that include both pharmacological and mechanical thrombolysis.
- *Pharmacological Thrombolysis*

  A microcatheter is positioned close to, or within and/or beyond the thrombus using an adjustable microguide. When the positioning of the microcatheter within the thrombus is not possible, the tip of the microcatheter must be placed as close as possible to the proximal surface of the thrombus, for local administration of Alteplase. A highly selective angiography through the microcatheter will be performed to show the correct positioning. The infusion of Alteplase will be started, at a dose of 90 mg/h, while the catheter will be gradually removed from the proximal surface of the thrombus. If recanalization is not obtained, the injection of potential vessel collateral may be necessary. Fibrinolytic therapy should be performed within 1 hour and the full dose of Alteplase infusion should not exceed 0.9 mg/Kg (max 90 mg in the case of body weight ≥ 100 Kg). If a complete recanalization is obtained, the Alteplase infusion can be interrupted before reaching the maximum dosage.

- *Mechanical thrombolysis*

  The option of performing a thrombolysis by mechanical means to obtain a mechanical disintegration/shift/detach/fissure of the thrombus and/or a retraction/aspiration can be considered on the basis of type, location and characteristics of the occlusion. This choice may simply involve the use of the microguidewire as a mechanical instrument to favor the disintegration of the thrombus, the use of systems to capture the thrombus by extraction, or more complex systems to crush and aspirate the

thrombus. The sophistication of some of these *devices* is noteworthy and requires specific *training*. This trial does not provide guide-lines for their use; the choice to use such devices is left to the experience and competence of the single health professional.

- *In the case of a negative angiography*
  In case of an angiography exam which shows no occlusion consistent to the symptomatology of the IA thrombolysis patient, the procedure will still be performed if the deficit is present; in the event that the occlusion is in a small vessel, Alteplase will be injected in that part of the vascular area that is presumably affected. If the patient shows no deficit, the administration of Alteplase is no longer indicated.
- *The case of residual stenosis*
  In case the resolution of the occlusion discloses the presence of a residual stenosis, treatment, where possible, is regarded as part of the procedure if the stenosis is considered directly responsible for residual clinical symptomatology. When the stenosis represents a recurrent thromboembolic risk factor, treatment, as secondary prevention in the acute phase, is left to the decision of the health professional.
- *IV Thrombolysis* and *bridging*
  For patients belonging to the IA group, IV thrombolysis could be considered in the following cases:
  - after randomization an obstacle or an estimation of delayed reaction to carry out the endovascular procedure occurs; in this case the health professional can decide whether to inject the whole dose of fibrinolytic or just a part (bridging):
  - selective catheterization is not practicable with a sufficient margin of safety;
  - a margin of benefit emerges, irrelevant of the selective intra-arterial injection of fibrinolytic, from the diagnostic re-evaluation with respect to the systemic intravenous one.

  Since the aim of the study is to compare IA and IV thrombolysis, the use of IV thrombolysis in randomized patients treated with IA is considered a violation of protocol.
- *Training in itinere*
  Participation to training courses *in itinere* is specifically required to health professionals and clinicians administering IA thrombolysis. The courses will be organized at the beginning and during the course of the study, offering discussion of cases or controversial issues encountered, guaranteeing a mutually uniform exposure of health professionals to the educational value of the contents that emerge and to the consistency of behavior adopted.

8.1.2 IV Thrombolysis

Thrombolytic treatment is started immediately after randomization, within 3 hours of symptoms onset. IV Alteplase is administered in a dose of 0.9 mg/Kg (max 90 mg), 10% of which is given as a bolus followed by delivery of the remaining 90% as a constant infusion over 60 minutes.

8.2 Associated therapies

All the patients in the two treatment groups will be given the most appropriate therapy.

- Antiplatelet therapy within 24 hours of symptoms onset should be avoided
- Low dose unfractioned heparin (5000 IU subcutaneous) or, preferably, low molecular weight heparin at prophylactic doses (4000 IU subcutaneous) may be used for patient at high risk of deep venous thrombosis (e.g. obesity and bed rest).
- Full-dose oral anticoagulant or, preferably, unfractioned heparin (e.g. to PT, INR > 1.5 for oral anticoagulant or aPTT >1.2 its normal value for unfractioned heparin) can be used in case of high-risk embolic sources (e.g. mechanical prosthetic valve), after exclusion of intracranial hemorrhage.
- The use of any antiplatelet or anticoagulant agent during the first week must be recorded in the CRF after randomization (Appendix B).
- All patients should be treated long term with an antiplatelet or oral agent, when indicated, for secondary prevention of stroke.

8.3 Protocol deviations

Whenever non expected treatments or procedures are used, such as drugs favoring recanalization, different from Alteplase (with the exception of heparin used during angiography), or the use of IV thrombolysis for patients in the IA group, they will be considered protocol deviations and will be recorded in the CRF after randomization.

9 POST RANDOMIZATION ASSESSMENT

After randomization information collection form

	Baseline	Follow up	
Time (days)	0 days	7 days stroke onset	90 days
	Randomization at treatment	During hospitalization *CRF after randomization*	During at home recovery *Telephone interview*

9.1 Assessment during hospital stay

The doctor following the patient during the hospital stay after randomization should fill in the online "CRF after randomization" (Appendix B) at 7 days from stroke onset, or at discharge or transfer to another hospital or death, depending on what occurs first. Completion of this part of the CRF also requires that the physician fill out the “Data available only to the doctor authorized to perform the blinded follow-up at 90 days" Appendix D, providing the patient's name and surname, full address, phone number, data of general practitioner and family members or people close to the patient. Access to such data will be allowed throughout the study exclusively to Dr. Anna Teresa Cantisani, Neurologist at the Silvestrini Hospital, Perugia, who will do the follow-up at 90 days, blinded to treatment allocation.

9.2 Long term assessment

Patient’s clinical conditions will be evaluated, by an expert examiner blinded to treatment allocation, by a telephone interview, 90 days after randomization (Dr. Anna Teresa Cantisani, Neurologist at the Silvestrini Hospital, Perugia). The examiner will use a check list of daily activities as a guide in questioning the patient (11,12). In case of unavailability of a patient, a proxy will be interviewed.
The blindness of the examiner will be verified for each patient assessed at 90 days.

The following aspects will be examined using the *modified Rankin score* divided into 6 categories:
0. *No symptoms*
1. *No significant disability despite symptoms*: able to carry out all usual duties and activities.
2. *Slight disability*: unable to carry out previous activities but able to look after own affairs without resistance.
3. *Moderate disability*: requiring some help, but able to walk without assistance.
4. *Moderately severe disability*: unable to walk without assistance and unable to attend to own bodily needs without assistance.
5. *Severe disability:* bedridden, incontinent and requiring constant nursing care and attention.
6. *Death*

The inter-observer agreement for differences of 2 grades on the modified Rankin scale is 0.91 (9) and its use by telephone instead of direct examination appears reliable (10).

New vascular episodes evaluated (recurrence of stroke, myocardial infarction, defining the diagnosis with the available information). Cause of death.

10. STATISTICAL METHODS

10.1. Calculation of sample size

The estimation of sample size for the primary *outcome* is based on a standard test of two samples per difference in binomial proportions (two-tailed test) with alpha = 5% and power = 80%. The study is to verify or refute an absolute difference of about 15% in the percentage of patients with a favorable outcome between the two treatment groups. At least 172 patients per arm should be enrolled, assuming that 40% of patients treated with IV Alteplase (estimate based on patients treated with Alteplase in the other *trial* (2) should produce a favorable outcome.

10.2 Statistical analysis

An "intention to treat" analysis will be used throughout the study. Analyses will be performed by the statistician blinded to treatment allocation that will be coded "A" or "B".
Analysis of the data relative to the 50 patients treated in the feasibility phase will remain separate and a *pooled analysis* will be performed between these patients and those relative to the expansion phase of the study. The protocol provides two separate analyses: primary analysis and secondary analysis.

10.2.1 Primary analysis

The primary analysis will evaluate the effect of IA thrombolysis compared to IV Alteplase on survival and autonomy at 90 days. Patients with modified Rankin scores of 0 or 1 are considered autonomous and non-autonomous or deceased the others (2, 3, 4, 5 or 6 on the modified Rankin scale). The statistical analysis will be conducted using the binary score of 0 or 1 of the Rankin scale as described above as the *endpoint* of the study. This score will be tabulated based on type of treatment (IV or IA). The result of cross-tabulation will be assessed with a two-tailed Fisher's exact test, in parallel with the calculation of Mantel-Haenszel ψ odds ratio and its confidence interval $\psi_{95\%}$.

10.2.2 Secondary analysis

Secondary analyses will include the following sub-analyses: a) the proportion of patients reaching an NIHSS score of $\leq$ 6 or less at day 7 following thrombolysis in the two treatment groups, with the Fisher's exact test; b) evaluation of the number of symptomatic intracranial hemorrhages, fatal and non-fatal strokes, deaths by any cause, cases of neurological deterioration, in both treatment groups, which will be compared with Fisher's exact test and binomial tests.
Subgroup analysis will then be carried out according to the main prognostic variables (age, severity of neurologic deficit, time elapsed between symptoms onset and randomization, CT results, atrial fibrillation, diabetes, hypertension and the etiopathologic classification of stroke).

All the variables of interest are subjected to exploratory graphical analysis to observe possible latent patterns. A descriptive analysis of each variable will then be made using average and standard deviation or median and range, on the basis of the particular distribution, which will be evaluated by the Shapiro-Wilk and Kolmogorov-Smirnov tests, used simultaneously. One variable will be considered of Gaussian type only if results are p> 0:05 in both tests.
The correlation matrix will be calculated later to evaluate possible relationships between the insistent independent variables, to judge the appropriateness of the simultaneous presence in the same multivariate generalized linear model (GLM). If a significant correlation between variables is found (assessed with the coefficient *r* followed by Fisher's exact test), the most biologically relevant or, in the case of further doubt or lack of clear biological hierarchy, the one which appears first in the timeline with respect to the event will be used.
The positive response (coded 0/1, as described above) will then be used as a categorical dependent variable in a set of GLM (both univariate and multivariate) with a matrix form of $\mathbf{y} = \mathbf{X\beta} + \boldsymbol{\varepsilon}$, where $\mathbf{y}$ is the vector of observed *endpoints*, $\mathbf{X}$ the design matrix, $\boldsymbol{\beta}$ the vector of unknown coefficients, and $\boldsymbol{\varepsilon}$ the vector of errors. Since the endpoint is binary, the logistic model appears most suitable for this purpose.
The models will be fitted with univariate analysis, which will select all possible *confounders* and regressors with results of $p<0.20$ on the Wald test. In an attempt to simplify the model for all

continuous variables, the GLM will be followed by a ROC (*Receiver Operating Characteristics*) for the detection of possible *cut-off* values of the variable itself, for which sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with respect to the *endpoint* will be determined; in addition to the absolute values the respective confidence intervals will also be calculated at 95%.
After selection of the most significant variables, multivariate models are fitted, the best of these will be selected on the basis of biological plausibility and by means of the following characteristics:
• likelihood ratio test (LR test) for the model, so that only the significant models will be taken into consideration,
• LR test for *nesting* models
• value of the pseudo-R_2 by McFadden, which will be used to estimate the variance explained by the model
• Wald test for each single regressor
Where there is an equivalence of plausibility between two or more models the most economic one will be considered.
The stepwise forward and backward method can be used for the selection of multivariate models.
Each model will be subjected to appropriate post-hoc diagnostics using *sensitivity analysis* and *goodness-of-fit* test in accordance with Hosmer and Lemeshow. Moreover, the plot of the experimental and model curves will be graphical, the values of VIF (variance inflating factor) for the independent variables will be calculated, and possible *outlier* among the diagonal elements of the Pregibon generalized hat matrix $\mathbf{H}_w$ will be found. The latter will eventually be identified by the analysis.

10.3 Further statistical considerations
Unless otherwise specified (see next paragraph), statistical significance will be utilized each time $p<0.05$, regardless of the test used. If the use of multiple tests for each *endpoint* becomes necessary, the threshold of significance will be reduced by using the standard Bonferroni criteria.
All calculations are performed using statistical software StataSE 10 (The Stata Corporation, College Station, TX), or a new release, if it were made available during the operations related to the protocol.

10.4 Interim safety and analysis
During the period of recruitment two interim analyses are planned, the first after the first 100 patients randomized and the second after the second 100 patients (i.e. the 200-th patient randomized).
It is assumed that the first error type in the first interim analysis is $\alpha_{i1} = 0.0001$ and in the second interim analysis $\alpha_{i2} = 0.001$, so that the cumulative error is equal to $\alpha_i = 0.0011$: thus, the final error of the first type becomes $\alpha_f = 0.049946$ (with target $\alpha_f < 0.05$), and therefore the statistical significance of the final test is employed only when starting from $p < 0.0489$.
The Safety and Monitoring Committee will suspend the study if an imbalance statistical and clinical in the relationship between risks and benefits is observed.

11. STUDY ORGANIZATION
The Scientific Committee of the study includes the following groups:

11.1 Steering and Organizing Committee
This committee is responsible for the design of the protocol and periodically re-evaluates the progression and operational level of the study. The committee supervises all relevant aspects regarding the progression and status of the study and is responsible for coordinating the clinical work as well as collecting and processing the data received from all the participating centers.

11.2 Participating centers
They are responsible for recruiting, treatment administered and data collection. The participating centers must have a stroke unit and an interventional neuroradiology department (see Appendix G of the requirements of participating centers).

11.3 Data management office

The Data management office has full responsibility for the study design, quality control and statistical analysis.
The correct use of inclusion criteria, therapeutic procedures and monitoring during hospitalization will be checked by a Clinical Monitor using the data provided in the electronic CRF of each single center. In case of contradictory, unclear or incorrect data, a telephone contact and/or visit to the center directly will be made, depending on the seriousness of the defect. In any case, the Clinical Monitor will make at least three direct visits to each center during the study.

11.4 Safety and Monitoring Committee

The committee is composed of permanent members, experienced neurologists and epidemiologists (Prof. Livia Candelise, University of Milan, Prof. Peter Sanderkock, University of Edinburgh, Professor Gregory del Zoppo, University of Washington in Seattle), who are not involved in carrying out the *trial*. The members of the group approve the final protocol, periodically reassess safety data on intercurrent events during hospitalization and carry out the two planned interim analysis. They may make relevant recommendations for the conduct of the study to the Steering and Organizing Committee.

12. ETHICAL ASPECTS

The trial will be initiated according to ICH Harmonized Tripartite for Good Clinical Practice guidelines and the Declaration of Helsinki and its subsequent amendments of Tokyo 1975, Venice 1983, Hong Kong 1989. The protocol is also in conformity with all relevant national and Community regulations applicable to clinical trials and to ethical and deontological principles that guide the medical practice. The approval of the Local Ethics Committee (or an equivalent) is required for each participating center before recruitment can begin.

12.1 Informed consent

Each patient will be given an information leaflet in support of informed consent. In general, the signature of informed consent is required. If the patient is unable to provide a written consent, the center coordinator should seek guidance from its ethics committee. It is considered ethically acceptable to record a verbal consent in the presence of a witness, if the patient is able to give consent but is unable to write, for example due to ipostenia of the hand, or apraxia or atassia [13,14]. If the patient appears cognitively unable to provide consent due to alterations of the upper functions as a result of stroke (i.e. aphasia, inattention, drowsiness), it will be obtained from the nearest available relative, [13,14]. If no relatives are available, exemption from informed consent can be obtained by following the guidelines of the U.S. Food and Drug Administration and the Department of Health and Human Services that allows hospitals to proceed without informed consent in critical situations of emergency [15].

13. CONTRIBUTIONS AND CONFLICT OF INTEREST

Dr. Alfonso Ciccone has conceived and written this protocol. Contributor to the paragraphs on statistics and randomization is Dr Michele Nichelatti, a specialist in medical statistics, Department of Oncology and Hematology, A.O. Niguarda Ca' Granda, and in the section on the description of intra-arterial thrombolysis Dr. Luca Valvassori, interventional neuroradiologist at the same hospital, and Dr. Francesco Scomazzoni, interventional neuroradiologist at the S. Raffaele Hospital in Milan.
No contributions have played a role in the preparation of this protocol.

14. SOURCES OF CONTRIBUTIONS

This trial was designed independently of any commercial organization and will be coordinated, managed and analyzed independently. The expansion of the study (SYNTHESIS Expansion) was made possible by a funding from the Italian Pharmaceutical Agency (AIFA).

15. FINAL REPORT AND PUBLICATION OF RESULTS

In agreement with the ICH-GCP, the Scientific Coordinator will undertake, in cooperation with investigators, to produce a Clinical Study Report, publish the findings arising from the clinical study as described in the Protocol and ensure that data are reported responsibly and consistently. It is understood that the results of the study will be disseminated by individual investigators, after agreement between the participating centers.

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APPENDIX A: CRF before randomization

(evaluation in ER)

Hospital _____ Province _____ Patient ID

Patient's personal data:

Family name and First name (initials)_____ ID _____
Date of birth (Day/Month/Year) ___/___/___ Sex (M/F) _____

Patient eligible for randomization **Yes No**

If yes, complete CRF
If not, fill in the form for patients eligible for thrombolysis within 3 hours but not randomized (link)

Timing:

Date of onset of stroke symptoms ___/___
Time of onset of stroke symptoms ___/___

Date of arrival at first Hospital ___/___
Time of arrival at first Hospital ___/___

Date of arrival at treating Hospital (if different from the first) ___/___
Time of arrival at treating Hospital (if different from the first) ___/___

Date of brain CT ___/___
Time of brain CT ___/___

Clinical Data:

Estimated body weight (Kg) ____

PAS upon arrival (mmHg) ___ PAD upon arrival (mmHg) ___

Atrial fibrillation upon arrival (ECG) **Yes No**

Antiplatelet/anticoagulant therapy in the previous 48 hours:
(tick one box on each line)

Any anticoagulant	**Yes** □ **No** □
Aspirin	**Yes** □ **No** □
Dypiridamole	**Yes** □ **No** □
Ibuprofen	**Yes** □ **No** □
Ticlopidine	**Yes** □ **No** □
Clopidogrel	**Yes** □ **No** □

Before admission for this stroke
(tick one box on each line)

Treatment for hypertension	**Yes** □ **No** □
Treatment for diabetes mellitus (insulin or other oral medications)	**Yes** □ **No** □
A history of previous stroke or TIA	**Yes** □ **No** □
A history of myocardial infarction	**Yes** □ **No** □
Did the patient live alone?	**Yes** □ **No** □
Was the patient independent in everyday activities?	**Yes** □ **No** □

Neurological deficit before randomization NIH STROKE SCALE

1a. Level of consciousness
0=Alert; keenly responsive.
1=Not alert; but arousable by minor stimulation to obey, answer, or respond. 2=Not alert; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped).
3=Responds only with reflex motor or autonomic effects, or totally unresponsive, flaccid, and areflexic.

1b. Level of Consciousness Questions:
0= Answers both questions correctly.
1= Answers one question correctly.
2= Answers neither question correctly.

1c. Level of Consciousness Commands:
0= Performs both tasks correctly.
1= Performs one task correctly.
2= Performs neither task correctly.

2. Best Gaze
0=Normal
2=Partial gaze palsy**;** gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present.
1= Forced deviation, or total gaze paresis is not overcome by the oculocephalic maneuver.

3. Visual
0= No visual loss.
1=Partial hemianopia
2=Complete hemianopia
3=Bilateral hemianopia or blindness

4. Facial Palsy
0= Normal symmetrical movements.
1= Minor paralysis (flattened nasolabial fold, asymmetry on smiling).
2= Partial paralysis (total or near-total paralysis of lower face).
3= Complete paralysis of one or both sides (absence of facial movement in the upper and lower face).

5. Left Motor Arm
0= No drift; limb holds 90 (or 45) degrees for full 10 seconds.
1= Drift; limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support.
2= Some effort against gravity; limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity.
3= No effort against gravity; limb falls.
4= No movement.
UN= Amputation or joint fusion.
5a. Left Arm→score
5b. Right Arm→score

6. Motor Leg
0=No drift; leg holds 30-degree position for full 5 seconds.
1=Drift; leg falls by the end of the 5-second period but does not hit the bed.
2=Some effort against gravity; leg falls to bed by 5 seconds but has some effort against gravity.
3=No effort against gravity; leg falls to bed immediately.
4=No movement.
UN=Amputation or joint fusion.
6a.Left Leg→score
6b. Right Leg→score

7.Limb Ataxia
0=Absent
1=Present in one limb.
2=Present in two limbs.
UN=Amputation or joint fusion.

8.Sensory
0=Normal; no sensory loss.
1=Mild-to-moderate sensory loss; patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched.
2=Severe or total sensory loss; patient is not aware of being touched in the face, arm, and leg.

9.Dysarthria
0=Normal.
1= Mild-to-moderate dysarthria; patient slurs at least some words and, at worst, can be understood with some difficulty.
2=Severe dysarthria; patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric.
UN=Intubated or other physical barrier.

10.Best Language
0=No aphasia; normal.
1= loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response.
2=Severe aphasia; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response.
3=Mute, global aphasia; no usable speech or auditory comprehension.

11.Extinction and Inattention (formerly Neglect)
0=No abnormality.
1=Visual, tactile, auditory, spatial, or personal inattention, or extinction to bilateral simultaneous stimulation in one of the sensory modalities.
2=Profound hemi-inattention or extinction to more than one modality; does not recognize own hand or orients to only one side of space.

TOTALE Score = _____ Date (Day/Month/Year) ___/___/___ **Hour/minutes** (24h)___/___

Inclusion criteria *(all must be answered yes)*

A) Sudden neurological deficit attributable to a cerebral stroke **Yes** □ **No** □
B) Age between 18 and 80 years **Yes** □ **No** □
C) Can start IV treatment within 3 hours **Yes** □ **No** □
D) Can start IA treatment within 6 hours **Yes** □ **No** □
E) The patient can be randomized within 3 hours **Yes** □ **No** □
F) Availability of an interventional neuroradiologist **Yes** □ **No** □

Exclusion criteria *(all must be answered no)*

- Severe stroke as assessed clinically (e.g. NIHSS>25) and/or adequate imaging techniques **Yes** □ **No** □
- Rapidly improving minor neurological deficit **Yes** □ **No** □
- Clinical presentation suggestive of a subarachnoid hemorrhage (even if CT scan is negative) **Yes** □ **No** □
- Seizure at onset of stroke **Yes** □ **No** □
- Coma at onset **Yes** □ **No** □
- Prior stroke within the last 3 months **Yes** □ **No** □
- Any history of prior stroke and concomitant diabetes mellitus **Yes** □ **No** □
- Major surgery or significant trauma in past 3 month **Yes** □ **No** □
- Recent or present acute or dangerous bleeding **Yes** □ **No** □
- Known hemorrhagic diathesis **Yes** □ **No** □
- Patients treated with oral anticoagulants **Yes** □ **No** □
- Administration of heparin within the previous 48 hours and a PTT exceeding the normal higher limit for the laboratory **Yes** □ **No** □
- Recent (>10 days) external heart massage, obstetrical delivery or puncture at a non compressible site (e.g. subclavian or jugular vein puncture) **Yes** □ **No** □
- Previous history of or suspected intracranial hemorrhage
- Previous history of central nervous system damage (neoplasm, aneurysm, intracranial surgery) **Yes** □ **No** □
- Documented ulcerative gastrointestinal disease in the last 3 months, esophageal varices **Yes** □ **No** □
- Severe liver disease, including hepatic failure, cirrhosis, portal hypertension (esophageal varices) and active hepatitis **Yes** □ **No** □
- Arterial aneurysm, vascular malformations **Yes** □ **No** □
- Neoplasm with increased bleeding risk **Yes** □ **No** □
- Bacterial endocarditis, pericarditis **Yes** □ **No** □
- Acute pancreatitis **Yes** □ **No** □
- Severe hypertension: PAS > 185 mmHg or PAD > 110 mm Hg uncontrolled or requiring continuous IV therapy **Yes** □ **No** □
- Baseline blood glucose < 50 mg per deciliter (2.75 mmol/L) or > 400 mg per deciliter (22mmol/L) **Yes** □ **No** □
- Platelet count < 100.000/mm^3 **Yes** □ **No** □
- Known contrast sensitivity **Yes** □ **No** □
- Women of childbearing potential or known to be breastfeeding **Yes** □ **No** □
- Prognosis very poor regardless of therapy (likely to be dead within months) **Yes** □ **No** □
- Disability preceding stroke (e.g., modified Rankin scale >1) **Yes** □ **No** □
- Unlikely to be available for follow-up (e.g., no fixed home address, visitor from overseas) **Yes** □ **No** □
- Refuses consent **Yes** □ **No** □
- Any other condition that the investigator believes may constitute a danger in terms of risk/benefit for the patient, or if the therapy is impracticable **Yes** □ **No** □

Computed tomographic (CT) scan exclusion criteria

- Hemorrhage of any degree **Yes** □ **No** □
- Intracranial tumors except small meningioma **Yes** □ **No** □
- Acute infarction (this may be an incorrect indicator of time of onset) **Yes** □ **No** □

Any further neuro-radiological examinations used to select patients:

Angio CT	**Yes** □ **No** □	Angio MR	**Yes** □ **No** □
Eco-Doppler TSA	**Yes** □ **No** □	TCCD	**Yes** □ **No** □
NMR diffusion	**Yes** □ **No** □	MRI perfusion	**Yes** □ **No** □
CT perfusion	**Yes** □ **No** □		

Informed consent modality
(tick only one box)
- Patient's signature □
- Patient's verbal consent □
- Assent by relative □
- Doctor's signature (consent/assent impossible) □

Treatment allocation
(tick only one box)
- IV Alteplase □
- IA Thrombolysis □

Date of randomization (day/month/year)___/___ /_____(automatic)
Time of randomization (24h)___/___ (automatic)

Doctor who performed randomization
Family name _____ Name _____

APPENDIX B: CRF- AFTER RANDOMIZATION

(to complete at 7 days, or discharge, or transfer to another hospital, or death, whichever occurs first)

Patient ID

Thrombolysis performed **Yes □ No □**
If *No*, specify _____

Thrombolysis interrupted early **Yes □ No □**
If *Yes*, specify_____

Total dose of Alteplase administered (mg):_____
N° batch of the drug: _____

Date of thrombolysis (day/month/year)___/___/___
Start time of therapy (24h)___/___
End time of therapy (24h)___/___
Start time of angiography (24h)___/___

Mechanical thrombolysis **Yes □ No □**
If yes, use of:
(Check only one box)
Angioplasty **Yes □ No □**
Fragmentation **Yes □ No □**
Embolectomy **Yes □ No □**
Aspiration **Yes □ No □**
Stent **Yes □ No □**
Other **Yes □ No □**
Type of *device* used (if used): _____

Deviation from protocol: specify if the following were used
- Antiplatelet within 24 hours of thrombolysis **Yes □ No □**
- IA Trombolysis in patients randomized to receive IV Alteplase **Yes □ No □**
- IV Alteplase (*bridging*) in patients randomized to receive IA Thrombolysis **Yes □ No □**
- Abciximab **Yes □ No □**
- Tirofiban **Yes □ No □**
- Other **Yes □ No □**

if yes, specify_____

Other therapies/procedures associated with thrombolysis
- IV Heparin **Yes □ No □**
- Sedation **Yes □ No □**
- Intubation **Yes □ No □**
- IV Hypotensive **Yes □ No □**
- Other **Yes □ No □**

If yes, specify _____

Therapies during hospitalization, after thrombolysis
- IV Glycerol **Yes □ No □**
- IV Mannitol **Yes □ No □**
- IV Furosemide **Yes □ No □**
- IV Labetalol **Yes □ No □**
- IV Nitroprusside **Yes □ No □**
- Low dose heparin/heparinoid (aPTT $\leq$1.2 fold normal value) **Yes □ No □**

- Full dose unfractioned heparin (aPTT > 1.2 fold normal value) **Yes** □ **No** □
- Full dose oral anticoagulants (INR > 1.5) **Yes** □ **No** □
- Aspirin **Yes** □ **No** □
- Any antiplatelet other than aspirin **Yes** □ **No** □

Control CT scan:
Date ___/___/___

Results of control CT scan:
(Check one box)
Normal □
Cerebral ischemia □
Hemorrhagic infarct □
Intracerebral hemorrhage □
Other intracranial hemorrhages □
Other □
If other, specify _____

Final diagnosis of the initial randomized event
(use all available clinical and/or radiological data)
(Check one box for each line)
Defined cerebral ischemia **Yes** □ **No** □
If yes, Specify localization:
- Anterior circulation **Yes** □ **No** □
- Posterior circulation **Yes** □ **No** □

Hemorrhagic infarct **Yes** □ **No** □
Non cerebrovascular event **Yes** □ **No** □
If yes,, Specify:
- Cerebral neoplasm **Yes** □ **No** □
- Migraine **Yes** □ **No** □
- Seizure **Yes** □ **No** □

Other **Yes** □ **No** □
If yes, specify:_____

Ethiologic diagnosis of defined cerebral ischemia
(Check ONLY one box)

- Large-artery atherosclerosis □
- Cardio embolic cerebral ischemia □
- Disease of the small vessels □
- Dissection □
- Other causes □
- Unknown causes □

EVENTS during hospitalization
(Check one box for each line)

Intra-angiographic complications	**Yes** □ **No** □	*If yes*, Specify date (day/month/year) ___/___/___
Hematoma at site of angiography injection	**Yes** □ **No** □	*If yes*, Specify date (day/month/year) ___/___/___
Lower limb ischemia	**Yes** □ **No** □	*If yes*, Specify date (day/month/year) ___/___/___
Symptomatic intracranial hemorrhage	**Yes** □ **No** □	*If yes*, Specify date (day/month/year) ___/___/___
New ischemic stroke	**Yes** □ **No** □	*If yes*, Specify date (day/month/year) ___/___/___
Cerebral edema	**Yes** □ **No** □	*If yes*, Specify date (day/month/year) ___/___/___
Mild extracranial bleeding	**Yes** □ **No** □	*If yes*, Specify date (day/month/year) ___/___/___
Severe extracranial bleeding	**Yes** □ **No** □	*If yes*, Specify date (day/month/year) ___/___/___
Myocardial infarction	**Yes** □ **No** □	*If yes*, Specify date (day/month/year) ___/___/___
Pulmonary thromboembolism	**Yes** □ **No** □	*If yes*, Specify date (day/month/year) ___/___/___
Pulmonary edema	**Yes** □ **No** □	*If yes*, Specify date (day/month/year) ___/___/___
Deep vein thrombosis	**Yes** □ **No** □	*If yes*, Specify date (day/month/year) ___/___/___
Anaphylactic shock	**Yes** □ **No** □	*If yes*, Specify date (day/month/year) ___/___/___
Death	**Yes** □ **No** □	*If yes*, Specify date (day/month/year) ___/___/___

Likely cause of death
(Check ONLY one box)

- Cerebral edema caused by the initial stroke, with or without evidence of intracranial bleeding □
- Recurrent stroke – type unknown □
- Intracranial hemorrhage □
- Extracranial hemorrhage □
- Heart attack □
- Sudden death □
- Pulmonary edema □
- Pulmonary thromboembolism □
- Pneumonia □
- Cause of death not specified □
- Other cause of death □

If another cause, specify: _____

If the patient is alive, complete the following parts after 7 days or when patient is discharged/moved to another Hospital, whichever happens first *(evaluate each point)*

Neurological deficit: NIH STROKE SCALE

1a. Level of consciousness

0=Alert; keenly responsive.

1=Not alert; but arousable by minor stimulation to obey, answer, or respond. 2=Not alert; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped).

3=Responds only with reflex motor or autonomic effects, or totally unresponsive, flaccid, and areflexic.

1b. Level of Consciousness Questions:

0= Answers both questions correctly.

1= Answers one question correctly.

2= Answers neither question correctly.

1c. Level of Consciousness Commands:

0= Performs both tasks correctly.

1= Performs one task correctly.

2= Performs neither task correctly.

2. Best Gaze

0=Normal

2=Partial gaze palsy**;** gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present.

1= Forced deviation, or total gaze paresis is not overcome by the oculocephalic maneuver.

3. Visual

0= No visual loss.

1=Partial hemianopia

2=Complete hemianopia

3=Bilateral hemianopia or blindness

4. Facial Palsy

0= Normal symmetrical movements.

1= Minor paralysis (flattened nasolabial fold, asymmetry on smiling).

2= Partial paralysis (total or near-total paralysis of lower face).

3= Complete paralysis of one or both sides (absence of facial movement in the upper and lower face).

5. Left Motor Arm

0= No drift; limb holds 90 (or 45) degrees for full 10 seconds.

1= Drift; limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support.

2= Some effort against gravity; limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity.

3= No effort against gravity; limb falls.

4= No movement.

UN= Amputation or joint fusion.

5a. Left Arm→score

5b. Right Arm→score

6. Motor Leg

0=No drift; leg holds 30-degree position for full 5 seconds.

1=Drift; leg falls by the end of the 5-second period but does not hit the bed.

2=Some effort against gravity; leg falls to bed by 5 seconds but has some effort against gravity.

3=No effort against gravity; leg falls to bed immediately.

4=No movement.

UN=Amputation or joint fusion.

6a.Left Leg→score

6b. Right Leg→score

7.Limb Ataxia
0=Absent
1=Present in one limb.
2=Present in two limbs.
UN=Amputation or joint fusion.

8.Sensory
0=Normal; no sensory loss.
1=Mild-to-moderate sensory loss; patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched.
2=Severe or total sensory loss; patient is not aware of being touched in the face, arm, and leg.

9.Dysarthria
0=Normal.
1= Mild-to-moderate dysarthria; patient slurs at least some words and, at worst, can be understood with some difficulty.
2=Severe dysarthria; patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric.
UN=Intubated or other physical barrier.

10.Best Language
0=No aphasia; normal.
1= loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response.
2=Severe aphasia; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response.
3=Mute, global aphasia; no usable speech or auditory comprehension.

11.Extinction and Inattention (formerly Neglect)
0=No abnormality.
1=Visual, tactile, auditory, spatial, or personal inattention, or extinction to bilateral simultaneous stimulation in one of the sensory modalities.
2=Profound hemi-inattention or extinction to more than one modality; does not recognize own hand or orients to only one side of space.

TOTALE Score = _____ Date (Day/Month/Year) ___/___/___ **HOURS/minutes** (24h)___/___

Fill out the form "Data accessible only to authorized doctors performing the follow-up at 90 days" (Link)

CRF Data compilation after randomization (Day/Month/Year) ___/___/___ (automatically)
CRF Hours compilation after randomization (24h)___/___ (automatically)

Doctor who performed CRF compilation after randomization
Last name _____ Name _____

Appendix C: Datasheet patients eligible for thrombolytic treatment within 3 hours but not randomized

Hospital _____ Province _____

Patient's personal data:

Last name and name (initials)_____ ID _____

Date of birth (Day/Month/Year) ___/___/___ Sex (M/F) _____

Date of stroke onset (Day/Month/Year) ___/___/___

Reasons for exclusion:

- ☐ Unavailable interventional neuroradiologist
- ☐ Angiography room not available
- ☐ Impossible to transport to angiography room
- ☐ Patient refuses consent
- ☐ Family members refuse consent
- ☐ Non-functional randomization system
- ☐ Important disability before stroke
- ☐ Other (specify)_____

APPENDIX D: Data accessible by the doctor authorized to carry out the follow-up at 90 days

(to be completed by the physician that fills out the post-randomisation CRF)

Hospital _____ Province _____

Patient's personal data:

Last name and name (initials)_____ ID _____
Date of birth (Day/Month/Year) ___/___/___ Sex (M/F) _____

Patient's complete address at discharge:

_____
_____
Zip code _____
Tel (home and mobile) _____

Data of family doctor:
Name of family doctor _____
Address of family doctor:_____
Zip code_____Tel._____

Indicate the name of person to contact if necessary
Nome:_____
Relationship: _____
Address:_____
Zip code_____Tel._____

Form filled out by_____
on (Day/Month/Year) ___/___/___

APPENDIX E: CRF Glossary

CT SCAN

Infarct Hypodense areas due to recent ischemic lesion in accordance with neurological deficit.
Hemorrhagic infarct One or more hyperdensity areas due to presence of blood, with speckled or mottled appearance and with indistinct margins, in the context of area of low attenuation representing infarction or edema.
Intracerebral hemorrhage Very dense, homogeneous region of increased density with distinct margins with or without mass effect including all or the major part of the infarcted lesion.
Other hemorrhages Intraparenchimal hemorrhage not related to the previous infarct or subdural hematoma or subarachnoid hemorrhage.

ETHIOLOGIC DIAGNOSIS

Cardioembolism: the arterial occlusion is presumably due to an embolus arising in the heart when there is one of the following high-risk cardiac source of embolism: mechanical prosthetic valve, atrial fibrillation with or without valvular heart disease, rheumatic mitral stenosis, atrial appendage thrombus, dilated cardiomiopathy, atrial mixoma, recent myocardial infarction with anterior wall infarction and/or akinetic segment and/or intraventricular thrombus. Diagnostic studies should exclude dissection as a possible cause of stroke.
Dissection: angiographic appearance of elongated and tapering stenosis, possibly with complete occlusion of the lumen and/or signs of intimal flap, a pseudoaneurysm (i.e. an aneurismal bulging of the adventitial wall to the false lumen) or a double lumen.
Large -artery atherosclerosis: angiographic findings of >50% stenosis or occlusion of a major brain artery or branch cortical artery, presumably due to atherosclerosis. A history of intermittent claudicatio, transient ischemic attacks (TIAs) in the same vascular territory, diminished pulses helps support the clinical diagnosis.
Diagnostic studies should exclude potential high-risk sources of cardiogenic embolism, dissection and other causes of stroke.
Disease of small vessels: evidence at control CT scan of subcortical infarcts ≤ 1.5cm in diameter or normal CT scan and a reasonable syndrome gap (motor stroke and/or pure sensory, hemiparesis, ataxia, in absence of disorders of the visual field, or a deficit due to higher nervous functions of new occurrence, or alterations in the brainstem, at the time of worst neurological deficit).
Other causes: diagnostic studies identify other ethiology such as: non-atherosclerotic vasculopaties, hypercoagulability states and hematological disorders. Diagnostic studies should exclude cardioembolism and dissection as possible cause of stroke.
Unknown causes: after excluding atherosclerotic, high-risk cardiac sources, dissection and other causes of thrombo-embolism.

Existing events during hospitalization and cause of death

Symptomatic intracranial hemorrhage Sudden neurological worsening after a period of stable condition or recovery, with documented intracranial hemorrhage (CT scan or autopsy). Neurological worsening is defined by one or more of the following:
1. any major change in the level of consciousness
2. any substantial change in degree of motor deficit
3. new deficits that are clinically significant and persistent

Extracranial bleeding It is classified as mild if bleeding did not required blood replacement, or as severe if requiring blood replacement.
New ichemic stroke Sudden neurological worsening (see intracranial hemorrhage) after a period of recovery or stable condition without documented intracranial hemorrhage or cerebral edema from the previous ischemic lesion.
Cerebral edema Neurological worsening, as described above, after a period of stable conditions or improvements in clinical conditions, due to the development of significant mass effect of the recent lesion, with midline shift.

Myocardial infarction At least two of the following: typical history, new appearance of abnormal Q waves on EKG, peak enzymes levels exceeding (twice the upper limit of normal).
Pulmonary thromboembolism Sudden appearance of dyspnea with or without chest pain. Suspect diagnosis must be confirmed by lung CT with contrast medium or autopsy.
Pulmonary edema Sudden appearance of dyspnea with aspiratory wheezing, in all lung fields, tachycardia, high blood pressure, urine contraction and chest x-ray compatible with lung congestion.
Anaphylactic shock Sudden respiratory distress with urticaria or angioedema followed by arterial hypotension (SBP < 90 mmHg) and oliguria (< 20 ml/hr) persisting for more than one hour, and within 12 hours of treatment.

APPENDIX F: Management during the first 7 days

Components of care after admission into the Hospital

- Bed rest progressing to full activity as tolerated:
- Care of bedridden patients
- Skin and joint care
- Bronchopulmonary care
- Watch for neurological worsening or hypotension during mobilization
- Measure vital and neurological signs:
- Neurological worsening
- Fever
- Hypertension or hypotension
- Cardiac monitoring during first 24 hours
- Assess swallowing before starting oral intake of fluids or solids; advance diet as tolerated
- Intravenous fluids to avoid dehydration
- Nasogastric tube feedings for patients who can not swallow
- Avoid indwelling bladder catheter if possible
- Symptomatic treatment of pain, nausea, agitation
- Treat medical or neurological complications
- Treat hearth disease and other co-morbid diseases
- Prophylaxis against deep-vein thrombosis

Acute treatment of hypertension

- Treat anxiety, pain, nausea, vomiting
- Treat increased intracranial pressure
- Do not acutely treat an elevated blood pressure
- If possible, give oral agents or reinstitute medications given before the stroke
- Gradually lower the blood pressure
- Monitor blood pressure at least every 30 minutes for two hours:

1. If systolic blood pressure is > 180 mmHg and/or diastolic blood pressure is 105 to 140 mmHg for two or more readings 5 to 10 minutes apart:

- Give intravenous Labetalol, 10 mg over 1 to 2 minutes in bolus.
- Monitor blood pressure every 15 minutes during Labetalol treatment and observe for development of hypotension.
- The dose of Labetalol may be repeated or doubled every 10 to 20 minutes up a total dose of 150 mg.

2. If diastolic blood pressure is > 140 mmHg for two separate readings 5 to 10 minutes apart or if the preceding treatment did not give satisfactory response:

- Infuse sodium Nitroprusside (0.5 to 10 mg/Kg/min).
- Monitor blood pressure every 15 minutes during infusion of sodium Nitroprusside and observe for development of hypotension.

If systolic blood pressure is <180 and/or diastolic blood pressure is <105 anti-hypertension treatment is usually discouraged. Conversely, hemorrhagic transformation requires a treatment of high blood pressure more aggressive than that outlined above because of the risk of continued bleeding or recurrent hemorrhage

Acute anticoagulant therapy

- Possible indications: high-risk source of embolism (e.g. mechanical prosthetic valve), pulmonary embolism, and "overt" deep vein thrombosis.
- Do not treat with full-dose oral anticoagulants or unfractionated heparin (e.g. to PT, INR > 1.5 with oral anticoagulant; to aPTT >1.2 fold normal with unfractionated heparin) if patient presents with ischemic lesion detectable by CT scan that is > 33% of the MCA territory, or any type of intracranial hemorrhage, unless the patient has a life-threatening condition (e.g. pulmonary embolus).

- Patients at high risk of deep venous thrombosis (e.g. plegia, obesity and obligated bed rest) should be placed on low dose unfractionated heparin (5000 units subcutaneous every 8 or 12 hours) or low molecular heparin (preferably) at prophylactic doses (4000 units subcutaneous). Otherwise physical prevention (e.g. pressure stockings and mobilization) is recommended.
- Intravenous unfractionated heparin should be initiated with weight based bolus infusion, and adjusted according to the weight-based nomogram until a therapeutic level (according to the aPTT) is reached.
- Acute anticoagulant treatment with unfractionated heparin usually requires an initial bolus injection of 5000-10,000 units followed by a continuous infusion of about 900 units/h or 10-15 units/Kg/h to maintain the PTT at 2-2.50 times that of the control time.

Treatment of increased intracranial pressure (brain edema, mass effect, hydrocephalus)

General prophylaxis

- Control fever, agitation, nausea and vomiting, hypoxia, hypercapnia
- Modest fluid restriction (approximately 1.5 L to 2 L/day)
- Avoid potential hypo-osmolar IV fluids
- Elevate the head of the bed to augment venous drainage (30°)

Acute interventions

- Mannitol 0.5 g/Kg given in a 18-20% solution over 20 to 30 minutes
- Can repeat 0.25 g/Kg every 6 hours as needed
- Usual maximal daily dose is 2g/Kg
- Replace lost fluids
- Furosemide 20 to 40 mg given IV

Treatment of intracranial hemorrhage following thrombolysis

- Stop any thrombolytic, anticoagulant or antiplatelet therapy
- Check hemoglobin, hematocrit, PT, aPTT, platelet count and fibrinogen
- Type and cross match 4 units of blood
- Give 4 to 6 units of cryoprecipitate to rise fibrinogen level to >150 mg/dl
- Recheck fibrinogen level every 4 hours and transfuse with cryoprecipitate to maintain fibrinogen level >150 mg/dl
- The hemostatic defect must be corrected before any surgery can be performed
- The blood pressure will need aggressive treatment because of the risk of continued bleeding or recurrent hemorrhage

APPENDIX G: Requirements of the participating centers

The participating centers must have a stroke unit and a department of interventional neuroradiology:
Stroke Unit: at least 4 beds dedicated to the care of stroke. Priority will be given to the centers that have participated to the SITS-MOST or that have experience in trials on thrombolysis.
Department of Interventional Neuroradiology: availability of working team in emergency, an anesthesiologist to assist the procedure if the patient requires sedation for treatment, interventional angiography operator, an angiography room nurse, a technician. The angiography operator must have experience in catheterisation of intracranial vessels and must have completed at least 10 endovascular treatment interventions (aneurysms, arteriovenous malformations, stent or thrombolysis).

SYNTHESIS Expansion

A randomized controlled trial between loco-regional intra-arterial (IA) and systemic intravenous (IV) thrombolysis with Alteplase in acute ischemic stroke

Prot. SYNTHESIS Expansion_07 – Version 2 - December 08

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1. SYNOPSIS

Title	A randomized controlled trial between loco-regional intra-arterial (IA) and systemic intravenous (IV) thrombolysis with Alteplase in acute ischemic stroke.
Study objectives	**Primary:** assess whether IA thrombolysis, as compared to the administration of Alteplase IV, increases survival free of disability (modified Rankin score of 0 or 1) at 3 months. **Secondary:** assess in the two treatment groups: • the neurological deficit 7 days after thrombolysis; • the safety of the procedure on the basis of events reported within 7 days following thrombolysis: symptomatic cerebral hemorrhage, fatal and non-fatal stroke, death from any cause, neurological deterioration.
Study design	Randomized, controlled, multicenter, open-label clinical trial with blinded follow-up. Phase III Study.
Study population	Patients with acute, symptomatic ischemic stroke, shown on CT, with the following conditions are eligible : 1. possibility of starting IV Alteplase treatment within 4 and a half hours from stroke onset; 2. possibility of starting IA thrombolysis within 6 hours from stroke onset; 3. uncertainty of the most appropriate choice.
Inclusion criteria	• Sudden focal neurological deficit attributable to a cerebral stroke • Clearly defined time of onset, allowing initiation of IV treatment within 4 and a half hours and IA treatment within 6 hours of symptoms onset. • Age between 18 and 80 years. • Availability of an interventional neuroradiologist.
Exclusion criteria	The exclusion criteria coincide exactly with those found in the Alteplase technical form *(see protocol for details)*. To those the following have been added: • Known contrast sensitivity; • Women of childbearing potential or known to be breastfeeding; • Prognosis very poor regardless of therapy (likely to be dead within months); • Disability preceding stroke (i.e. modified Rankin scale of >1); • Unlikely to be available for follow-up (i.e. no fixed home address, visitor from overseas); • Refused consent; • Any other condition which the investigator feels could pose a hazard in terms of risk/benefit to the patient, or if the therapy proves impracticable.
Computed tomographic (CT) scan exclusion criteria	• Intracranial tumors, except small meningioma. • Hemorrhage of any degree. • Acute infarction (since this may be an indicator that the time of symptoms onset is not correct).
Treatment plans	**Experimental arm:** IA thrombolysis with Alteplase up to 0.9 mg/Kg (max 90 mg), administered with microcatheters, introduced preferably at the femoral artery, and/or with mechanical devices (fragmentation/retraction/aspiration). The neuroradiologist may choose the procedure most appropriate to the circumstances and administer a lower dose of IA Alteplase than the maximum allowed, in case of re-canalization of the vessel. The IA procedure must start as soon as possible and no later than 6 hours from stroke onset.

	Control arm: Alteplase in a dose of 0.9 mg/kg of body weight (maximum 90 mg), administered as intravenous infusion over 60 minutes, 10% of which is given as an initial intravenous bolus.
Calculation of sample size	The estimation of sample size for the primary *outcome* is based on a standard test of two samples for differences in binomial proportions (two-tailed test) with alpha = 5% and power = 80%. The study seeks to verify or refute an absolute difference of about 15% in the percentage of patients with a favorable *outcome* between the two treatment groups. At least 172 patients per arm should be enrolled, assuming that 40% of patients treated with IV Alteplase (estimate is based on patients treated with Alteplase in other studies) should provide a favorable *outcome*.
Statistical analysis	"Intention to treat" analyses will be used throughout the study. The analyses will be made by a statistician, blinded with respect to treatment which will be coded "A" or "B". The protocol will have two separate analyses: primary analysis and secondary analysis. Primary analysis will assess the effect of IA thrombolyis compared to IV Alteplase on survival and autonomy after 90 days. Those patients with modified Rankin scores of 0 or 1 are considered self-sufficient, the others are disabled or dead (2, 3, 4, 5 or 6 on the modified Rankin scale). Statistical analysis will be carried out using a 0 to 1 track score of the Rankin scale noted above as the *endpoint* of the study. This score will be tabulated by type of treatment (IA or IV). The result of the cross-tabulation will be assessed with a two tailed exact Fisher test, in parallel with calculation of the Mantel-Haenszel odds ratio ψ and the confidence interval ψ95%. Secondary analysis includes the following sub-analysis: a) analysis of the proportion of patients reaching an NIHSS score of ≤ 6 at day seven after thrombolysis, which will be conducted with Fisher's exact test; 2) assessment of the number of symptomatic intracranial hemorrhages, fatal and non-fatal strokes, death from any cause, neurological deterioration, in the two treatment groups, which will be compared with Fisher's exact test and binomial test. Subgroups analyses will then be conducted according to main baseline prognostic variables (age, severity of neurological deficit, time to randomization from stroke onset, CT scan, arterial fibrillation, diabetes mellitus, hypertension and the causes of stroke) *(see protocol)*.
Safety and interim analysis	During the period of recruitment two interim analyses are planned: the first after the first 100 patients randomized and the second after the second 100 patients (which means after 200 patients randomized). The Safety and Monitoring Committee will suspend the study if a statistical and a clinical imbalance in the risk to benefit ratio is found.
Study duration	Beginning recruitment date : February 2008 End recruitment date: April 2012.

2. RATIONALE

Stroke is a major public health concern in Italy, where its incidence is approximately 155.000 new cases (and 39.000 recurrences) per year. It represents the third cause of death after cardiovascular and neoplastic diseases, and is the cause of 10-12% of all deaths/year. Acute (30 days) mortality for stroke has been evaluated to be equal to 20% of all cases in Italy, while during the first year it is quantifiable as 30%. One year after stroke, one third of the surviving subjects show an elevated degree of disability, sufficient to define them as totally dependent (1).

There is evidence that IV thrombolysis is the only effective treatment to work in ischemic stroke within the first few hours of symptoms onset so far. Risks and benefits of thrombolysis are summarized in the systematic review of randomized controlled trials (RCTs) produced by The Cochrane Collaboration: thanks to thrombolytic therapy with IV Altaplase, which is approved for the treatment of ischemic stroke within three hours from onset in several countries, 103 more patients were alive and independent at the end of follow up for every 1000 patients treated (2). A successive meta-analysis demonstrated that IV Altaplase remains effective in the therapeutic window between 3 and 4 and a half hours, though in a lesser measure compared to when it is administered within the first 3 hours of symptoms onset (3). These data were recently confirmed by the results of an ad hoc RCT on patients treated between 3 and 4 and a half hours (4).

For over 25 years few interventional neuroradiologists have been successfully treating this category of patients by an IA route. However, evidence is still required to support the clinical feeling that IA treatment, which needs longer time and greater complexity, indeed leads to a better outcome with respect to the IV approach (5, 6).

- *Pros and cons of IA thrombolysis*

  IA thrombolysis might offer many advantages over the IV route: such as being able to titer the dosage of the thrombolytic agent, to ensure a high drug concentration locally and low concentration in the systemic circulation, to facilitate recanalization with mechanical thrombolysis, to extend the therapeutic time window. However, compared to IV thrombolysis, the IA strategy requires more advanced technology and human resources and is consequently limited to highly specialized centers. Moreover, IA thrombolysis not only implies an expertise in neuro-intervention but also an organization that requires the prompt availability of a neuro-interventionist, a stroke team and a consolidated fast track to the angiography room.

- *Proof of effectiveness*

  The Cochrane review (2) identified only two RCTs on IA thrombolysis for acute ischemic stroke: PROACT(7) and PROACT II (8). Both studies compared the use of recombinant pro-urokinase (r-pro-UK) plus IA heparin or IV heparin only in patients with middle cerebral artery occlusion, randomized within 6 hours from symptoms onset. The meta-analysis of the two trials shows that IA treatment increases the risk of symptomatic intracranial hemorrhage (absolute risk increment 7% - a similar increase is observed with IV Alteplase,) but reduces the percentage of patients dead or dependent at long-term (absolute risk reduction 13%). However, this confidence interval was wide, due to the small sample size, and included the possibility that IA treatment might prevent even 1 dead or dependent patient per 100 treated. After the last update of the Cochrane review, another two RCTs were published on IA thrombolysis. In a first study, 16 patients with stroke and angiographic evidence of posterior circulation occlusion were randomized to be treated within 24 hours after stroke onset with IA UK or no treatment (control). All patients were acutely anti-coagulated. Four of eight patients who received IA UK compared with seven of the eight in the control group were dead or disabled at six months and there were four deaths in each treatment group. This small RCT, which was stopped before reaching the 200 patients planned on, due to slow recruitment and withdrawal of UK from the market, was definitely underpowered, and the small difference in outcome between the two groups may be explained by the play of chance. The second study (10), compared IV UK with IA UK administered within 6 hours of symptoms onset, was also stopped early because of 7 deaths: 4 in the group of 14 with IV treatment and 3 in the group of 13 treated with IA. Although the patients treated with IA in this study saw early improvement and to a higher degree, there was no difference in primary and secondary *outcomes* between the two groups.

- *Conclusion*
  Evidence on acute stroke management with IA thrombolysis is still scarce; there is only one RCT which compares the two approaches (IA and IV), stopped early. Moreover, previous RCTs were aimed to assess the efficacy of a specific thrombolytic drug rather than the complete IA approach. Indeed, patients were randomized after angiography (i.e. angiography and its associated risks were not considered as an integral part of the IA approach) and the IA procedure was strictly standardized (for instance mechanical devices were forbidden).
- *Innovation of Synthesis compared to previous studies*
  Synthesis is a pragmatic multicentric RCT that takes into account the above mentioned issues in order to compare the IA and IV in clinical practice:
  1. inclusion and exclusion criteria are based on those used for IV treatment with Alteplase;
  2. patients are considered for the study when uncertainty about appropriateness of the two approaches exists;
  3. patients are randomized before angiography, which is considered an integral part of the IA approach;
  4. patients randomized to IV Alteplase are treated within 4 and a half hours from stroke onset;
  5. patients randomized to IA thrombolysis are to be treated as soon as possible but can be treated also after 4 and a half hours from symptoms onset (but never more than 6 hours) as the time taken for IA approach is considered an integral part of treatment;
  6. IA thrombolysis can be both pharmacological and mechanical (the procedural choices of the interventional neuroradiologist depend on the type of occlusion, circumstances and personal experience);
  7. the sample size was planned considering a consistent superiority of IA over IV thrombolysis, to justify the use of a complex procedure that requires an increase in the amount of resources.
- *Possible impact of IA thrombolysis in clinical practice*
  The number of centers able to perform IA thrombolysis is definitely inadequate compared to the burden of patients with stroke but is probably sufficient to collaborate in a multicenter trial to clarify the role of IA thrombolysis for acute stroke management. The effort is worthy of a trial because a positive result could justify an allocation of resources in this direction and more widespread use of this treatment. Indeed, if IA thrombolysis proves so effective compared to IV thrombolysis then it should become available for most people, not just for the few lucky enough to be admitted to a specialist tertiary referral center.

3. START-UP/FEASIBILITY PHASE AND EXPANSION PHASE

The present expansion phase of the *SYNTHESIS* study, called *SYNTHESIS Expansion*, follows the *SYNTHESIS* study, which began in January 2004 is still on-going and will be concluded with the start of the present protocol. The *SYNTHESIS* study, which involved the Department of Neurosciences of the Niguarda Ca' Granda Hospital as coordinating center and 3 other centers in the Lombard region (*Spedali Civili* in Brescia, *S. Raffaele* Hospital in Milan, *Valduce* Hospital in Como), demonstrated the feasibility of the study. The feasibility phase was monitored by the Safety and Monitoring Committee composed of Prof. Livia Candelise, University of Milan, Prof. Peter Sanderkock, University of Edinburgh, and Prof. Gregory del Zoppo, University of Washington in Seattle.

The present expansion phase of the study intends to involve at least 10-20 centers with experience in endovascular interventions and equipped with a Stroke Unit, able to recruit 350 patients over a two year period. The *SYNTHESIS Expansion* protocol has been modified with respect to the earlier *SYNTHESIS*. The most relevant modification consists in having expanded the possibilities of endovascular interventions, leaving ample discretion in the choice of the approach to use. Therefore, the study is no longer a comparison between IV Alteplase and IA Alteplase but rather between IV Alteplase and endovascular intervention. In short, the crucial question to be answered by the study is: if it is better to trust a patient to the interventional neuroradiologist rather than treat him/her with

IV Alteplase, according to a pragmatic approach which reflects what actually happens and the modifications of recent years in the field of endovascular intervention. The interventional neuroradiologist is therefore free to use any mechanical *device* to splinter, melt or extract the thrombus.

4. OBJECTIVES

4.1 Primary aims

To assess whether local IA thrombolysis, as compared to IV Alteplase, increases survival free of disability (modified Rankin score of zero or 1) at 3 months.

4.2 Secondary aims

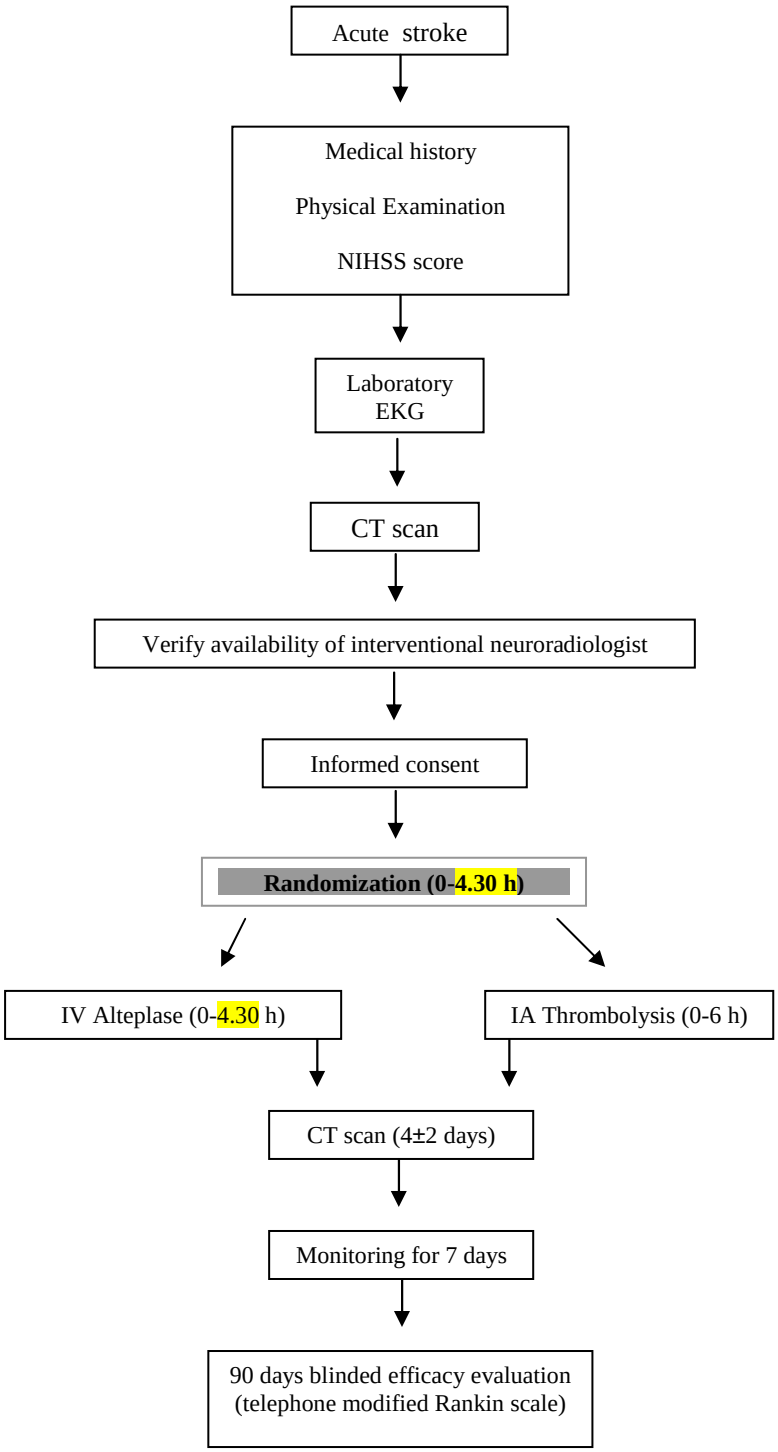
To assess in the two treatment groups:

1. the neurological deficit 7 day after thrombolysis;
2. the safety of the procedure on the basis of events reported within 7 days after thrombolysis: symptomatic intracranial hemorrhages, fatal and non-fatal stroke, death from any cause, neurological deterioration.

5. STUDY DESIGN

SYNTHESIS Expansion is a randomized, controlled, multicenter, open-label clinical trial with blinded follow-up, that proposes to verify if IA thrombolysis, as compared to IV thrombolysis with Alteplase, within 4 and a half hours from ischemic stroke onset, increases the number of autonomous patients at 90 days.

5.1 Study outline - FLOW CHART



6. PATIENT ELIGIBILITY

Patients with acute, symptomatic ischemic stroke, shown on CT, with the following conditions are eligible :

1. possibility of starting IV Alteplase treatment within 4 and a half hours from stroke onset;
2. possibility of starting IA thrombolysis within 6 hours from stroke onset;
3. uncertainty of the most appropriate choice.

6.1 Clinical inclusion criteria

- Sudden focal neurological deficit attributable to a cerebral stroke.
- Clearly defined time of onset, allowing initiation of IV treatment within 4 and a half hours and IA treatment within 6 hours of symptoms onset.
- Age between 18 and 80 years.
- Availability of an interventional neuroradiologist.

6.2 Clinical and laboratory exclusion criteria

- Severe stroke as assessed clinically (e.g. NIHSS>25) and/or adequate imaging techniques
- Rapidly improving minor neurological deficit
- Clinical presentation suggestive of a subarachnoid hemorrhage (even if CT scan is negative)
- Seizure at onset of stroke
- Coma at onset
- Prior stroke within the last 3 months
- Any history of prior stroke and concomitant diabetes mellitus
- Major surgery or significant trauma in past 3 month
- Recent or present acute or dangerous bleeding
- Known hemorrhagic diathesis
- Patients in treatment with oral anticoagulants and prolonged PT (INR > 1.6)
- Administration of heparin within the previous 48 hours and a PTT exceeding the normal higher limit for the laboratory
- Recent (<10 days) external heart massage, obstetrical delivery or puncture at a non compressible site (e.g. subclavian or jugular vein puncture)
- Previous history of or suspected intracranial hemorrhage
- Previous history of central nervous system damage (neoplasm, aneurysm, intracranial surgery)
- Documented ulcerative gastrointestinal disease in the last 3 months, esophageal varices
- Severe liver disease, including hepatic failure, cirrhosis, portal hypertension (esophageal varices) and active hepatitis
- Arterial aneurysm, vascular malformations
- Neoplasm with increased bleeding risk
- Bacterial endocarditis, pericarditis
- Acute pancreatitis
- Severe hypertension: PAS >185 mmHg or PAD >110 mm Hg uncontrolled or requiring continuous IV therapy
- Baseline blood glucose <50 mg per deciliter (2.75 mmol/L) or >400 mg per deciliter (22mmol/L)
- Platelet count < 100.000/mm^3
- Known contrast sensitivity
- Women of childbearing potential or known to be breastfeeding
- Prognosis very poor regardless of therapy (likely to be dead within months)
- Disability preceding stroke (e.g., modified Rankin scale >1)
- Unlikely to be available for follow-up (e.g., no fixed home address, visitor from overseas)
- Refuses consent

- Any other condition which investigators feel would pose a significant hazard in terms of risk/benefit to the patient, or if therapies are impracticable.

6.3 Computed tomographic (CT) scan exclusion criteria

- Intracranial tumors except small meningiomas
- Hemorrhage of any degree
- Acute infarction (this may be an incorrect indicator of time of onset)

6.4 Principles of treatment uncertainties and patient selection

The researcher must be uncertain as to the best treatment to administer (IA or IV) to be able to randomize. To make a therapeutic decision, the possibility of considering non invasive examinations to visualize the occlusion (such as MR angiography, CT angiography or Doppler US) or the compromised cerebral area (diffusion MRI studies and/or perfusion CT/MRI studies) is not excluded. However, as there are uncertainties as to the use of patient selection in thrombolytic therapies with the above mentioned methods, these exams have not been considered essential for this study. They can therefore be used at the discretion of the researcher provided their use is noted in the CRF before randomization (Appendix A).

6.5 NON RANDOMIZED ELIGIBLE PATIENTS

A register will be kept for non-randomized patients, which will include the patient's initials, sex, age, date of observation, and reason for exclusion. (CRF "Form for non-randomized patients eligible for treatment with thrombolysis within 4 and a half hours" Appendix C). These patients will be not followed-up.

7. RANDOMIZATION

The study provides for a simple randomization that will be carried out *on line* in a centralized way after filling out, again *on line*, data from the "CRF before randomization" (Appendix A). Randomization will be performed by the neurologist or emergency doctor who visits the patient in the emergency room. It will be done with hardware and software techniques, using a computer with a GNU/Linux operating system, equipped with connections to a series of radio devices (www.random.org). These will be tuned to different frequencies (where there are no artificial signals), making it possible to pick-up white sounds generated by the atmosphere. The noise is made into a sample with 8 bit and 8khz signal algorithms, that are filtered and converted into sequence of binary digits to high entropy.

The numbers generated are tested according to the recommendations of the U.S. National Institute of Standards and Technology (NIST). In particular, before being assigned to a treatment or another (IA Alteplase or Alteplase IV), the sequence 0/1 of the bits of randomization will be tested with the calculation of the integral

$$\operatorname{erfc}\left(\frac{|S|}{\sqrt{2}}\right) = p = \frac{2}{S\sqrt{2}} \int_{x}^{3} e^{-t^2} dt$$

in wich $S = b_1 + b_2 + \ldots + b_n$ is the sum of the sequence of bits. The sequence will be accepted as be random if p <0.05.

8. STUDY TREATMENTS

The study provides a comparison of the two treatments:

- *Experimental treatment (IA thrombolysis):* Alteplase to 0.9 mg/kg (max 90 mg), administered by IA, by means of a microcatheter preferably introduced via femoral artery and/or mechanical thrombolysis (fragmentation/retraction/aspiration). The neuroradiologist has the possibility of choosing the procedure that best suits the situation and of administering a dose of IA Alteplase less than the maximum allowed, in case of recanalization of the vessel. The IA procedure must start as rapidly as possible and no later than 6 hours from stroke onset.
- *Standard treatment (IV thrombolysis):* the recommended dose is 0.9 mg of Alteplase/kg body weight (max 90 mg) administered as an intravenous infusion, 10% of which is given as a bolus followed by delivery of the remaining 90% as a constant infusion over 60 minutes.

8.1 Description of study procedures

8.1.1 IA Thrombolysis

- *Premise*

  IA procedure can vary according to circumstances, type of occlusion and experience of the health professional. While taking this into account, it is necessary to establish a certain homogeneity in IA treatment between the different participating units, both through the indications that follow and the continuous exchange of information and experience among researchers in the study. Variations in procedure are allowed as long as they are recorded in the in-hospital evaluation form.
- *Timing*

  The IA procedure must be performed as soon as possible after randomization and, in any case, within 6 hours from symptoms onset (taking into account possible impediments such as difficulty of catheterization or the need for anesthetics).
- *Anesthetic assistance*

  It is recommended that the availability of anesthetic assistance be evaluated at randomization. The need for anesthetic sedation in order to carry out a procedure is discretionary and must be reported in the CRF after randomization (Appendix B).
- *Cerebral arteriography*

  Arteriography precedes the therapeutic phase and must be targeted to acquiring data essential for making the endovascular therapeutic choices.

  Anticoagulant therapy, although recommended, is performed at the discretion of the health professional according to the current standard adopted in the single centers, it must be reported in the CRF after randomization (Appendix B). If anticoagulant therapy is used, an initial administration of 5000 IU of IV heparin in bolus is recommended, followed by 500IU/h infusion until the conclusion of the angiography.

  Once the diagnostic information has been acquired (site of occlusion, cerebral circulation, collaterals), the health professional can consider different therapeutic strategies that include both pharmacological and mechanical thrombolysis.
- *Pharmacological Thrombolysis*

  A microcatheter is positioned close to, or within and/or beyond the thrombus using an adjustable microguide. When the positioning of the microcatheter within the thrombus is not possible, the tip of the microcatheter must be placed as close as possible to the proximal surface of the thrombus, for local administration of Alteplase. A highly selective angiography through the microcatheter will be performed to show the correct positioning. The infusion of Alteplase will be started, at a dose of 90 mg/h, while the catheter will be gradually removed from the proximal surface of the thrombus. If recanalization is not obtained, the injection of potential vessel collateral may be necessary. Fibrinolytic therapy should be performed within 1 hour and the full dose of Alteplase infusion should not exceed 0.9 mg/Kg (max 90 mg in the case of body weight ≥ 100 Kg). If a complete recanalization is obtained, the Alteplase infusion can be interrupted before reaching the maximum dosage.

- *Mechanical thrombolysis*
  The option of performing a thrombolysis by mechanical means to obtain a mechanical disintegration/shift/detach/fissure of the thrombus and/or a retraction/aspiration can be considered on the basis of type, location and characteristics of the occlusion. This choice may simply involve the use of the microguidewire as a mechanical instrument to favor the disintegration of the thrombus, the use of systems to capture the thrombus by extraction, or more complex systems to crush and aspirate the thrombus. The sophistication of some of these *devices* is noteworthy and requires specific *training*. This trial does not provide guide-lines for their use; the choice to use such devices is left to the experience and competence of the single health professional.
- *In the case of a negative angiography*
  In case of an angiography exam which shows no occlusion consistent to the symptomatology of the IA thrombolysis patient, the procedure will still be performed if the deficit is present; in the event that the occlusion is in a small vessel, Alteplase will be injected in that part of the vascular area that is presumably affected. If the patient shows no deficit, the administration of Alteplase is no longer indicated.
- *The case of residual stenosis*
  In case the resolution of the occlusion discloses the presence of a residual stenosis, treatment, where possible, is regarded as part of the procedure if the stenosis is considered directly responsible for residual clinical symptomatology. When the stenosis represents a recurrent thromboembolic risk factor, treatment, as secondary prevention in the acute phase, is left to the decision of the health professional.
- *IV Thrombolysis* and *bridging*
  For patients belonging to the IA group, IV thrombolysis could be considered in the following cases:
  - after randomization an obstacle or an estimation of delayed reaction to carry out the endovascular procedure occurs; in this case the health professional can decide whether to inject the whole dose of fibrinolytic or just a part (bridging):
  - selective catheterization is not practicable with a sufficient margin of safety;
  - a margin of benefit emerges, irrelevant of the selective intra-arterial injection of fibrinolytic, from the diagnostic re-evaluation with respect to the systemic intravenous one.

  Since the aim of the study is to compare IA and IV thrombolysis, the use of IV thrombolysis in randomized patients treated with IA is considered a violation of protocol.
- *Training in itinere*
  Participation to training courses *in itinere* is specifically required to health professionals and clinicians administering IA thrombolysis. The courses will be organized at the beginning and during the course of the study, offering discussion of cases or controversial issues encountered, guaranteeing a mutually uniform exposure of health professionals to the educational value of the contents that emerge and to the consistency of behavior adopted.

8.1.2 IV Thrombolysis

Thrombolytic treatment is started immediately after randomization, within 4 and a half hours of symptoms onset. IV Alteplase is administered in a dose of 0.9 mg/Kg (max 90 mg), 10% of which is given as a bolus followed by delivery of the remaining 90% as a constant infusion over 60 minutes.

8.2 Associated therapies

All the patients in the two treatment groups will be given the most appropriate therapy.

- Antiplatelet therapy within 24 hours of symptoms onset should be avoided
- Low dose unfractioned heparin (5000 IU subcutaneous) or, preferably, low molecular weight heparin at prophylactic doses (4000 IU subcutaneous) may be used for patient at high risk of deep venous thrombosis (e.g. obesity and bed rest).
- Full-dose oral anticoagulant or, preferably, unfractioned heparin (e.g. to PT, INR > 1.5 for oral anticoagulant or aPTT >1.2 its normal value for unfractioned heparin) can be used in case of high-risk embolic sources (e.g. mechanical prosthetic valve), after exclusion of intracranial hemorrhage.

- The use of any antiplatelet or anticoagulant agent during the first week must be recorded in the CRF after randomization (Appendix B).
- All patients should be treated long term with an antiplatelet or oral agent, when indicated, for secondary prevention of stroke.

8.3 Protocol deviations

Whenever non expected treatments or procedures are used, such as drugs favoring recanalization, different from Alteplase (with the exception of heparin used during angiography), or the use of IV thrombolysis for patients in the IA group, they will be considered protocol deviations and will be recorded in the CRF after randomization.

9 POST RANDOMIZATION ASSESSMENT

After randomization information collection form

	Baseline	Follow up	
Time (days)	0 days	7 days stroke onset	90 days
	Randomization at treatment	During hospitalization *CRF after randomization*	During at home recovery *Telephone interview*

9.1 Assessment during hospital stay

The doctor following the patient during the hospital stay after randomization should fill in the online "CRF after randomization" (Appendix B) at 7 days from stroke onset, or at discharge or transfer to another hospital or death, depending on what occurs first. Completion of this part of the CRF also requires that the physician fill out the “Data available only to the doctor authorized to perform the blinded follow-up at 90 days" Appendix D, providing the patient's name and surname, full address, phone number, data of general practitioner and family members or people close to the patient. Access to such data will be allowed throughout the study exclusively to Dr. Anna Teresa Cantisani, Neurologist at the Silvestrini Hospital, Perugia, who will do the follow-up at 90 days, blinded to treatment allocation.

9.2 Long term assessment

Patient’s clinical conditions will be evaluated, by an expert examiner blinded to treatment allocation, by a telephone interview, 90 days after randomization (Dr. Anna Teresa Cantisani, Neurologist at the Silvestrini Hospital, Perugia). The examiner will use a check list of daily activities as a guide in questioning the patient (11,12). In case of unavailability of a patient, a proxy will be interviewed.
The blindness of the examiner will be verified for each patient assessed at 90 days.

The following aspects will be examined using the *modified Rankin score* divided into 6 categories:
0. *No symptoms*
1. *No significant disability despite symptoms*: able to carry out all usual duties and activities.
2. *Slight disability*: unable to carry out previous activities but able to look after own affairs without resistance.
3. *Moderate disability*: requiring some help, but able to walk without assistance.
4. *Moderately severe disability*: unable to walk without assistance and unable to attend to own bodily needs without assistance.
5. *Severe disability:* bedridden, incontinent and requiring constant nursing care and attention.
6. *Death*

The inter-observer agreement for differences of 2 grades on the modified Rankin scale is 0.91 (12) and its use by telephone instead of direct examination appears reliable (11).

New vascular episodes evaluated (recurrence of stroke, myocardial infarction, defining the diagnosis with the available information). Cause of death.

10. ASSESSMENT OF NEUROIMAGES

All patients included in the study, must be given a brain CT scan before randomization. A control CT scan is scheduled for the 4th day (± 2). Treatment for patients randomized to the IA group is performed under angiography. There is also the possibility of performing non-invasive examinations to visualize the occluded vessel (CT angiography/MR angiography) or the cerebral area affected (MRI diffusion studies e/o perfusion studies with CT/MRI) when deciding therapy.

For each single randomized patient in the study, the images of neuroradiological CT/MR analyses and angiographic procedures in those patients randomized to the IA arm of treatment, will be sent by post in a CD format to the Coordinating Center of the Synthesis Expansion study, where they will be made anonymous for the archives and reading by the Central Neuroradiology Committee.

11. STATISTICAL METHODS

11.1. Calculation of sample size

The estimation of sample size for the primary *outcome* is based on a standard test of two samples per difference in binomial proportions (two-tailed test) with alpha = 5% and power = 80%. The study is to verify or refute an absolute difference of about 15% in the percentage of patients with a favorable outcome between the two treatment groups. At least 172 patients per arm should be enrolled, assuming that 40% of patients treated with IV Alteplase (estimate based on patients treated with Alteplase in the other *trial*2) should produce a favorable outcome.

11.2 Statistical analysis

An “intention to treat” analysis will be used throughout the study. Analyses will be performed by the statistician blinded to treatment allocation that will be coded “A” or “B”.
Analysis of the data relative to the 50 patients treated in the feasibility phase will remain separate and a *pooled analysis* will be performed between these patients and those relative to the expansion phase of the study. The protocol provides two separate analyses: primary analysis and secondary analysis.

11.2.1 Primary analysis

The primary analysis will evaluate the effect of IA thrombolysis compared to IV Alteplase on survival and autonomy at 90 days. Patients with modified Rankin scores of 0 or 1 are considered autonomous and non-autonomous or deceased the others (2, 3, 4, 5 or 6 on the modified Rankin scale). The statistical analysis will be conducted using the binary score of 0 or 1 of the Rankin scale as described above as the *endpoint* of the study. This score will be tabulated based on type of treatment (IV or IA). The result of cross-tabulation will be assessed with a two-tailed Fisher’s exact test, in parallel with the calculation of Mantel-Haenszel ψ odds ratio and its confidence interval $\psi_{95\%}$.

11.2.2 Secondary analysis

Secondary analyses will include the following sub-analyses: a) the proportion of patients reaching an NIHSS score of $\leq$ 6 or less at day 7 following thrombolysis in the two treatment groups, with the Fisher’s exact test; b) evaluation of the number of symptomatic intracranial hemorrhages, fatal and non-fatal strokes, deaths by any cause, cases of neurological deterioration, in both treatment groups, which will be compared with Fisher's exact test and binomial tests.
Subgroup analysis will then be carried out according to the main prognostic variables (age, severity of neurologic deficit, time elapsed between symptoms onset and randomization, CT results, atrial fibrillation, diabetes, hypertension and the etiopathologic classification of stroke).

All the variables of interest are subjected to exploratory graphical analysis to observe possible latent patterns. A descriptive analysis of each variable will then be made using average and standard deviation or median and range, on the basis of the particular distribution, which will be

evaluated by the Shapiro-Wilk and Kolmogorov-Smirnov tests, used simultaneously. One variable will be considered of Gaussian type only if results are p> 0:05 in both tests.
The correlation matrix will be calculated later to evaluate possible relationships between the insistent independent variables, to judge the appropriateness of the simultaneous presence in the same multivariate generalized linear model (GLM). If a significant correlation between variables is found (assessed with the coefficient *r* followed by Fisher's exact test), the most biologically relevant or, in the case of further doubt or lack of clear biological hierarchy, the one which appears first in the timeline with respect to the event will be used.
The positive response (coded 0/1, as described above) will then be used as a categorical dependent variable in a set of GLM (both univariate and multivariate) with a matrix form of $\mathbf{y} = \mathbf{X\beta} + \boldsymbol{\varepsilon}$, where **y** is the vector of observed *endpoints*, **X** the design matrix, **β** the vector of unknown coefficients, and **ε** the vector of errors. Since the endpoint is binary, the logistic model appears most suitable for this purpose.
The models will be fitted with univariate analysis, which will select all possible *confounders* and regressors with results of $p<0.20$ on the Wald test. In an attempt to simplify the model for all continuous variables, the GLM will be followed by a ROC (*Receiver Operating Characteristics*) for the detection of possible *cut-off* values of the variable itself, for which sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with respect to the *endpoint* will be determined; in addition to the absolute values the respective confidence intervals will also be calculated at 95%.
After selection of the most significant variables, multivariate models are fitted, the best of these will be selected on the basis of biological plausibility and by means of the following characteristics:

- likelihood ratio test (LR test) for the model, so that only the significant models will be taken into consideration,
- LR test for *nesting* models
- value of the pseudo-R_2 by McFadden, which will be used to estimate the variance explained by the model
- Wald test for each single regressor

Where there is an equivalence of plausibility between two or more models the most economic one will be considered.
The stepwise forward and backward method can be used for the selection of multivariate models.
Each model will be subjected to appropriate post-hoc diagnostics using *sensitivity analysis* and *goodness-of-fit* test in accordance with Hosmer and Lemeshow. Moreover, the plot of the experimental and model curves will be graphical, the values of VIF (variance inflating factor) for the independent variables will be calculated, and possible *outlier* among the diagonal elements of the Pregibon generalized hat matrix $\mathbf{H}_w$ will be found. The latter will eventually be identified by the analysis.

11.3 Further statistical considerations

Unless otherwise specified (see next paragraph), statistical significance will be utilized each time $p<0.05$, regardless of the test used. If the use of multiple tests for each *endpoint* becomes necessary, the threshold of significance will be reduced by using the standard Bonferroni criteria.
All calculations are performed using statistical software StataSE 10 (The Stata Corporation, College Station, TX), or a new release, if it were made available during the operations related to the protocol.

11.4 Interim safety and analysis

During the period of recruitment two interim analyses are planned, the first after the first 100 patients randomized and the second after the second 100 patients (i.e. the 200-th patient randomized).
It is assumed that the first error type in the first interim analysis is $\alpha_{i1} = 0.0001$ and in the second interim analysis $\alpha_{i2} = 0.001$, so that the cumulative error is equal to $\alpha_i = 0.0011$: thus, the final error of the first type becomes $\alpha_f = 0.049946$ (with target $\alpha_f < 0.05$), and therefore the statistical significance of the final test is employed only when starting from $p < 0.0489$.
The Safety and Monitoring Committee will suspend the study if an imbalance statistical and clinical in the relationship between risks and benefits is observed.

12. STUDY ORGANIZATION

The Scientific Committee of the study includes the following groups:

12.1 Steering and Organizing Committee

This committee is responsible for the design of the protocol and periodically re-evaluates the progression and operational level of the study. The committee supervises all relevant aspects regarding the progression and status of the study and is responsible for coordinating the clinical work as well as collecting and processing the data received from all the participating centers.

12.2 Participating centers

They are responsible for recruiting, treatment administered and data collection. The participating centers must have a stroke unit and an interventional neuroradiology department (see Appendix G of the requirements of participating centers).

12.3 Data management office

The Data management office has full responsibility for the study design, quality control and statistical analysis.

The correct use of inclusion criteria, therapeutic procedures and monitoring during hospitalization will be checked by a Clinical Monitor using the data provided in the electronic CRF of each single center. In case of contradictory, unclear or incorrect data, a telephone contact and/or visit to the center directly will be made, depending on the seriousness of the defect. In any case, the Clinical Monitor will make at least three direct visits to each center during the study.

12.4 Safety and Monitoring Committee

The committee is composed of permanent members, experienced neurologists and epidemiologists (Prof. Livia Candelise, University of Milan, Prof. Peter Sanderkock, University of Edinburgh, Professor Gregory del Zoppo, University of Washington in Seattle), who are not involved in carrying out the *trial*. The members of the group approve the final protocol, periodically reassess safety data on intercurrent events during hospitalization and carry out the two planned interim analysis. They may make relevant recommendations for the conduct of the study to the Steering and Organizing Committee.

12.5 Neuroradiology comittee

The Neuroradiology Committee is composed of experts in the field neuroradiology: interventional neurovascular and cerebrovascular diseases. This committee contributes to the formation and homogeneity of interventions involved in the study and re-evaluates the neuro-imaging of the randomized patients.

13. ETHICAL ASPECTS

The trial will be initiated according to ICH Harmonized Tripartite for Good Clinical Practice guidelines and the Declaration of Helsinki and its subsequent amendments of Tokyo 1975, Venice 1983, Hong Kong 1989. The protocol is also in conformity with all relevant national and Community regulations applicable to clinical trials and to ethical and deontological principles that guide the medical practice. The approval of the Local Ethics Committee (or an equivalent) is required for each participating center before recruitment can begin.

13.1 Informed consent

Each patient will be given an information leaflet in support of informed consent. In general, the signature of informed consent is required. If the patient is unable to provide a written consent, the center coordinator should seek guidance from its ethics committee. It is considered ethically acceptable to record a verbal consent in the presence of a witness, if the patient is able to give consent but is unable to write, for example due to ipostenia of the hand, or apraxia or atassia [13,14]. If the patient appears cognitively unable to provide consent due to alterations of the upper functions as a result of stroke (i.e. aphasia, inattention, drowsiness), it will be obtained from the nearest available relative, [13,14]. If no relatives are available, exemption from informed consent can be obtained by following the guidelines of the U.S. Food and Drug Administration and the Department of Health and Human Services that allows hospitals to proceed without informed consent in critical situations of emergency [15].

14. CONTRIBUTIONS AND CONFLICT OF INTEREST

Dr. Alfonso Ciccone has conceived and written this protocol. Contributor to the paragraphs on statistics and randomization is Dr Michele Nichelatti, a specialist in medical statistics, Department of Oncology and Hematology, A.O. Niguarda Ca' Granda, and in the section on the description of intra-arterial thrombolysis Dr. Luca Valvassori, interventional neuroradiologist at the same hospital, and Dr. Francesco Scomazzoni, interventional neuroradiologist at the S. Raffaele Hospital in Milan.
No contributions have played a role in the preparation of this protocol.

15. SOURCES OF CONTRIBUTIONS

This trial was designed independently of any commercial organization and will be coordinated, managed and analyzed independently. The expansion of the study (SYNTHESIS Expansion) was made possible by a funding from the Italian Pharmaceutical Agency (AIFA).

16. FINAL REPORT AND PUBLICATION OF RESULTS

In agreement with the ICH-GCP, the Scientific Coordinator will undertake, in cooperation with investigators, to produce a Clinical Study Report, publish the findings arising from the clinical study as described in the Protocol and ensure that data are reported responsibly and consistently.
It is understood that the results of the study will be disseminated by individual investigators, after agreement between the participating centers.

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APPENDIX A: CRF before randomization

(evaluation in ER)

Hospital _____ Province _____ Patient ID

Patient's personal data:

Family name and First name (initials)_____ ID _____
Date of birth (Day/Month/Year) ___/___/___ Sex (M/F) _____

Patient eligible for randomization **Yes No**

If yes, complete CRF
If not, fill in the form for patients eligible for thrombolysis within 4 and a half hours but not randomized (link)

Timing:

Date of onset of stroke symptoms ___/___
Time of onset of stroke symptoms ___/___

Date of arrival at first Hospital ___/___
Time of arrival at first Hospital ___/___

Date of arrival at treating Hospital (if different from the first) ___/___
Time of arrival at treating Hospital (if different from the first) ___/___

Date of brain CT ___/___
Time of brain CT ___/___

Clinical Data:

Estimated body weight (Kg) ____

PAS upon arrival (mmHg) ___ PAD upon arrival (mmHg) ___

Atrial fibrillation upon arrival (ECG) **Yes No**

Antiplatelet/anticoagulant therapy in the previous 48 hours:
(tick one box on each line)

Any anticoagulant	**Yes** □ **No** □
Aspirin	**Yes** □ **No** □
Dypiridamole	**Yes** □ **No** □
Ibuprofen	**Yes** □ **No** □
Ticlopidine	**Yes** □ **No** □
Clopidogrel	**Yes** □ **No** □

Before admission for this stroke
(tick one box on each line)

Treatment for hypertension	**Yes** □ **No** □
Treatment for diabetes mellitus (insulin or other oral medications)	**Yes** □ **No** □
A history of previous stroke or TIA	**Yes** □ **No** □
A history of myocardial infarction	**Yes** □ **No** □
Did the patient live alone?	**Yes** □ **No** □
Was the patient independent in everyday activities?	**Yes** □ **No** □

Neurological deficit before randomization NIH STROKE SCALE

1a. Level of consciousness
0=Alert; keenly responsive.
1=Not alert; but arousable by minor stimulation to obey, answer, or respond. 2=Not alert; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped).
3=Responds only with reflex motor or autonomic effects, or totally unresponsive, flaccid, and areflexic.

1b. Level of Consciousness Questions:
0= Answers both questions correctly.
1= Answers one question correctly.
2= Answers neither question correctly.

1c. Level of Consciousness Commands:
0= Performs both tasks correctly.
1= Performs one task correctly.
2= Performs neither task correctly.

2. Best Gaze
0=Normal
2=Partial gaze palsy**;** gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present.
1= Forced deviation, or total gaze paresis is not overcome by the oculocephalic maneuver.

3. Visual
0= No visual loss.
1=Partial hemianopia
2=Complete hemianopia
3=Bilateral hemianopia or blindness

4. Facial Palsy
0= Normal symmetrical movements.
1= Minor paralysis (flattened nasolabial fold, asymmetry on smiling).
2= Partial paralysis (total or near-total paralysis of lower face).
3= Complete paralysis of one or both sides (absence of facial movement in the upper and lower face).

5. Left Motor Arm
0= No drift; limb holds 90 (or 45) degrees for full 10 seconds.
1= Drift; limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support.
2= Some effort against gravity; limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity.
3= No effort against gravity; limb falls.
4= No movement.
UN= Amputation or joint fusion.
5a. Left Arm→score
5b. Right Arm→score

6. Motor Leg
0=No drift; leg holds 30-degree position for full 5 seconds.
1=Drift; leg falls by the end of the 5-second period but does not hit the bed.
2=Some effort against gravity; leg falls to bed by 5 seconds but has some effort against gravity.
3=No effort against gravity; leg falls to bed immediately.
4=No movement.
UN=Amputation or joint fusion.
6a.Left Leg→score
6b. Right Leg→score

7.Limb Ataxia
0=Absent
1=Present in one limb.
2=Present in two limbs.
UN=Amputation or joint fusion.

8.Sensory
0=Normal; no sensory loss.
1=Mild-to-moderate sensory loss; patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched.
2=Severe or total sensory loss; patient is not aware of being touched in the face, arm, and leg.

9.Dysarthria
0=Normal.
1= Mild-to-moderate dysarthria; patient slurs at least some words and, at worst, can be understood with some difficulty.
2=Severe dysarthria; patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric.
UN=Intubated or other physical barrier.

10.Best Language
0=No aphasia; normal.
1= loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response.
2=Severe aphasia; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response.
3=Mute, global aphasia; no usable speech or auditory comprehension.

11.Extinction and Inattention (formerly Neglect)
0=No abnormality.
1=Visual, tactile, auditory, spatial, or personal inattention, or extinction to bilateral simultaneous stimulation in one of the sensory modalities.
2=Profound hemi-inattention or extinction to more than one modality; does not recognize own hand or orients to only one side of space.

TOTALE Score = _____ Date (Day/Month/Year) ___/___/___ **Hour/minutes** (24h)___/___

Inclusion criteria *(all must be answered yes)*

A) Sudden neurological deficit attributable to a cerebral stroke **Yes** □ **No** □
B) Age between 18 and 80 years **Yes** □ **No** □
C) Can start IV treatment within 4 and a half hours **Yes** □ **No** □
D) Can start IA treatment within 6 hours **Yes** □ **No** □
E) The patient can be randomized within 4 and a half hours **Yes** □ **No** □
F) Availability of an interventional neuroradiologist **Yes** □ **No** □

Exclusion criteria *(all must be answered no)*

- Severe stroke as assessed clinically (e.g. NIHSS>25) and/or adequate imaging techniques **Yes** □ **No** □
- Rapidly improving minor neurological deficit **Yes** □ **No** □
- Clinical presentation suggestive of a subarachnoid hemorrhage (even if CT scan is negative) **Yes** □ **No** □
- Seizure at onset of stroke **Yes** □ **No** □
- Coma at onset **Yes** □ **No** □
- Prior stroke within the last 3 months **Yes** □ **No** □
- Any history of prior stroke and concomitant diabetes mellitus **Yes** □ **No** □
- Major surgery or significant trauma in past 3 month **Yes** □ **No** □
- Recent or present acute or dangerous bleeding **Yes** □ **No** □
- Known hemorrhagic diathesis **Yes** □ **No** □
- Patients treated with oral anticoagulants and INR>1.6 **Yes** □ **No** □
- Administration of heparin within the previous 48 hours and a PTT exceeding the normal higher limit for the laboratory **Yes** □ **No** □
- Recent (>10 days) external heart massage, obstetrical delivery or puncture at a non compressible site (e.g. subclavian or jugular vein puncture) **Yes** □ **No** □
- Previous history of or suspected intracranial hemorrhage
- Previous history of central nervous system damage (neoplasm, aneurysm, intracranial surgery) **Yes** □ **No** □
- Documented ulcerative gastrointestinal disease in the last 3 months, esophageal varices **Yes** □ **No** □
- Severe liver disease, including hepatic failure, cirrhosis, portal hypertension (esophageal varices) and active hepatitis **Yes** □ **No** □
- Arterial aneurysm, vascular malformations **Yes** □ **No** □
- Neoplasm with increased bleeding risk **Yes** □ **No** □
- Bacterial endocarditis, pericarditis **Yes** □ **No** □
- Acute pancreatitis **Yes** □ **No** □
- Severe hypertension: PAS > 185 mmHg or PAD > 110 mm Hg uncontrolled or requiring continuous IV therapy **Yes** □ **No** □
- Baseline blood glucose < 50 mg per deciliter (2.75 mmol/L) or > 400 mg per deciliter (22mmol/L) **Yes** □ **No** □
- Platelet count < 100.000/mm^3 **Yes** □ **No** □
- Known contrast sensitivity **Yes** □ **No** □
- Women of childbearing potential or known to be breastfeeding **Yes** □ **No** □
- Prognosis very poor regardless of therapy (likely to be dead within months) **Yes** □ **No** □
- Disability preceding stroke (e.g., modified Rankin scale >1) **Yes** □ **No** □
- Unlikely to be available for follow-up (e.g., no fixed home address, visitor from overseas) **Yes** □ **No** □
- Refuses consent **Yes** □ **No** □
- Any other condition that the investigator believes may constitute a danger in terms of risk/benefit for the patient, or if the therapy is impracticable **Yes** □ **No** □

Computed tomographic (CT) scan exclusion criteria

- Hemorrhage of any degree **Yes** □ **No** □
- Intracranial tumors except small meningioma **Yes** □ **No** □
- Acute infarction (this may be an incorrect indicator of time of onset) **Yes** □ **No** □

Any further neuro-radiological examinations used to select patients:

Angio CT	**Yes** □ **No** □	Angio MR	**Yes** □ **No** □
Eco-Doppler TSA	**Yes** □ **No** □	TCCD	**Yes** □ **No** □
NMR diffusion	**Yes** □ **No** □	MRI perfusion	**Yes** □ **No** □
CT perfusion	**Yes** □ **No** □		

Informed consent modality
(tick only one box)
- Patient's signature □
- Patient's verbal consent □
- Assent by relative □
- Doctor's signature (consent/assent impossible) □

Treatment allocation
(tick only one box)
- IV Alteplase □
- IA Thrombolysis □

Date of randomization (day/month/year)___/___ /_____(automatic)
Time of randomization (24h)___/___ (automatic)

Doctor who performed randomization
Family name _____ Name _____

APPENDIX B: CRF- AFTER RANDOMIZATION

(to complete at 7 days, or discharge, or transfer to another hospital, or death, whichever occurs first)

Patient ID

Thrombolysis performed **Yes** □ **No** □
If *No*, specify _____

Thrombolysis interrupted early **Yes** □ **No** □
If *Yes*, specify_____

Total dose of Alteplase administered (mg):_____
N° batch of the drug: _____

Date of thrombolysis (day/month/year)___/___/___
Start time of therapy (24h)___/___
End time of therapy (24h)___/___
Start time of angiography (24h)___/___

Mechanical thrombolysis **Yes** □ **No** □
If yes, use of:
(Check only one box)
Angioplasty **Yes** □ **No** □
Fragmentation **Yes** □ **No** □
Embolectomy **Yes** □ **No** □
Aspiration **Yes** □ **No** □
Stent **Yes** □ **No** □
Other **Yes** □ **No** □
Type of *device* used (if used): _____

Deviation from protocol: specify if the following were used
- Antiplatelet within 24 hours of thrombolysis **Yes** □ **No** □
- IA Trombolysis in patients randomized to receive IV Alteplase **Yes** □ **No** □
- IV Alteplase (*bridging*) in patients randomized to receive IA Thrombolysis **Yes** □ **No** □
- Abciximab **Yes** □ **No** □
- Tirofiban **Yes** □ **No** □
- Other **Yes** □ **No** □

if yes, specify_____

Other therapies/procedures associated with thrombolysis
- IV Heparin **Yes** □ **No** □
- Sedation **Yes** □ **No** □
- Intubation **Yes** □ **No** □
- IV Hypotensive **Yes** □ **No** □
- Other **Yes** □ **No** □

If yes, specify _____

Therapies during hospitalization, after thrombolysis
- IV Glycerol **Yes** □ **No** □
- IV Mannitol **Yes** □ **No** □
- IV Furosemide **Yes** □ **No** □
- IV Labetalol **Yes** □ **No** □
- IV Nitroprusside **Yes** □ **No** □
- Low dose heparin/heparinoid (aPTT $\leq$1.2 fold normal value) **Yes** □ **No** □

- Full dose unfractioned heparin (aPTT > 1.2 fold normal value) **Yes** □ **No** □
- Full dose oral anticoagulants (INR > 1.5) **Yes** □ **No** □
- Aspirin **Yes** □ **No** □
- Any antiplatelet other than aspirin **Yes** □ **No** □

Control CT scan:
Date ___/___/___

Results of control CT scan:
(Check one box)
Normal □
Cerebral ischemia □
Hemorrhagic infarct □
Intracerebral hemorrhage □
Other intracranial hemorrhages □
Other □
If other, specify _____

Final diagnosis of the initial randomized event
(use all available clinical and/or radiological data)
(Check one box for each line)
Defined cerebral ischemia **Yes** □ **No** □
If yes, Specify localization:
- Anterior circulation **Yes** □ **No** □
- Posterior circulation **Yes** □ **No** □

Hemorrhagic infarct **Yes** □ **No** □
Non cerebrovascular event **Yes** □ **No** □
If yes,, Specify:
- Cerebral neoplasm **Yes** □ **No** □
- Migraine **Yes** □ **No** □
- Seizure **Yes** □ **No** □

Other **Yes** □ **No** □
If yes, specify:_____

Ethiologic diagnosis of defined cerebral ischemia
(Check ONLY one box)

- Large-artery atherosclerosis ☐
- Cardio embolic cerebral ischemia ☐
- Disease of the small vessels ☐
- Dissection ☐
- Other causes ☐
- Unknown causes ☐

EVENTS during hospitalization
(Check one box for each line)

Intra-angiographic complications	**Yes** ☐ **No** ☐	*If yes*, Specify date (day/month/year) ___/___/___
Hematoma at site of angiography injection	**Yes** ☐ **No** ☐	*If yes*, Specify date (day/month/year) ___/___/___
Lower limb ischemia	**Yes** ☐ **No** ☐	*If yes*, Specify date (day/month/year) ___/___/___
Symptomatic intracranial hemorrhage	**Yes** ☐ **No** ☐	*If yes*, Specify date (day/month/year) ___/___/___
New ischemic stroke	**Yes** ☐ **No** ☐	*If yes*, Specify date (day/month/year) ___/___/___
Cerebral edema	**Yes** ☐ **No** ☐	*If yes*, Specify date (day/month/year) ___/___/___
Mild extracranial bleeding	**Yes** ☐ **No** ☐	*If yes*, Specify date (day/month/year) ___/___/___
Severe extracranial bleeding	**Yes** ☐ **No** ☐	*If yes*, Specify date (day/month/year) ___/___/___
Myocardial infarction	**Yes** ☐ **No** ☐	*If yes*, Specify date (day/month/year) ___/___/___
Pulmonary thromboembolism	**Yes** ☐ **No** ☐	*If yes*, Specify date (day/month/year) ___/___/___
Pulmonary edema	**Yes** ☐ **No** ☐	*If yes*, Specify date (day/month/year) ___/___/___
Deep vein thrombosis	**Yes** ☐ **No** ☐	*If yes*, Specify date (day/month/year) ___/___/___
Anaphylactic shock	**Yes** ☐ **No** ☐	*If yes*, Specify date (day/month/year) ___/___/___
Death	**Yes** ☐ **No** ☐	*If yes*, Specify date (day/month/year) ___/___/___

Likely cause of death
(Check ONLY one box)

- Cerebral edema caused by the initial stroke, with or without evidence of intracranial bleeding ☐
- Recurrent stroke – type unknown ☐
- Intracranial hemorrhage ☐
- Extracranial hemorrhage ☐
- Heart attack ☐
- Sudden death ☐
- Pulmonary edema ☐
- Pulmonary thromboembolism ☐
- Pneumonia ☐
- Cause of death not specified ☐
- Other cause of death ☐

If another cause, specify: _____

If the patient is alive, complete the following parts after 7 days or when patient is discharged/moved to another Hospital, whichever happens first *(evaluate each point)*

Neurological deficit: NIH STROKE SCALE

1a. Level of consciousness
0=Alert; keenly responsive.
1=Not alert; but arousable by minor stimulation to obey, answer, or respond. 2=Not alert; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped).
3=Responds only with reflex motor or autonomic effects, or totally unresponsive, flaccid, and areflexic.

1b. Level of Consciousness Questions:
0= Answers both questions correctly.
1= Answers one question correctly.
2= Answers neither question correctly.

1c. Level of Consciousness Commands:
0= Performs both tasks correctly.
1= Performs one task correctly.
2= Performs neither task correctly.

2. Best Gaze
0=Normal
2=Partial gaze palsy**;** gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present.
1= Forced deviation, or total gaze paresis is not overcome by the oculocephalic maneuver.

3. Visual
0= No visual loss.
1=Partial hemianopia
2=Complete hemianopia
3=Bilateral hemianopia or blindness

4. Facial Palsy
0= Normal symmetrical movements.
1= Minor paralysis (flattened nasolabial fold, asymmetry on smiling).
2= Partial paralysis (total or near-total paralysis of lower face).
3= Complete paralysis of one or both sides (absence of facial movement in the upper and lower face).

5. Left Motor Arm
0= No drift; limb holds 90 (or 45) degrees for full 10 seconds.
1= Drift; limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support.
2= Some effort against gravity; limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity.
3= No effort against gravity; limb falls.
4= No movement.
UN= Amputation or joint fusion.
5a. Left Arm→score
5b. Right Arm→score

6. Motor Leg
0=No drift; leg holds 30-degree position for full 5 seconds.
1=Drift; leg falls by the end of the 5-second period but does not hit the bed.
2=Some effort against gravity; leg falls to bed by 5 seconds but has some effort against gravity.
3=No effort against gravity; leg falls to bed immediately.
4=No movement.
UN=Amputation or joint fusion.
6a.Left Leg→score
6b. Right Leg→score

7.Limb Ataxia
0=Absent
1=Present in one limb.
2=Present in two limbs.
UN=Amputation or joint fusion.

8.Sensory
0=Normal; no sensory loss.
1=Mild-to-moderate sensory loss; patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched.
2=Severe or total sensory loss; patient is not aware of being touched in the face, arm, and leg.

9.Dysarthria
0=Normal.
1= Mild-to-moderate dysarthria; patient slurs at least some words and, at worst, can be understood with some difficulty.
2=Severe dysarthria; patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric.
UN=Intubated or other physical barrier.

10.Best Language
0=No aphasia; normal.
1= loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response.
2=Severe aphasia; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response.
3=Mute, global aphasia; no usable speech or auditory comprehension.

11.Extinction and Inattention (formerly Neglect)
0=No abnormality.
1=Visual, tactile, auditory, spatial, or personal inattention, or extinction to bilateral simultaneous stimulation in one of the sensory modalities.
2=Profound hemi-inattention or extinction to more than one modality; does not recognize own hand or orients to only one side of space.

TOTALE Score = _____ Date (Day/Month/Year) ___/___/___ **HOURS/minutes** (24h)___/___

Fill out the form "Data accessible only to authorized doctors performing the follow-up at 90 days" (Link)

CRF Data compilation after randomization (Day/Month/Year) ___/___/___ (automatically)
CRF Hours compilation after randomization (24h)___/___ (automatically)

Doctor who performed CRF compilation after randomization
Last name _____ Name _____

Appendix C: Datasheet patients eligible for thrombolytic treatment within 4 hours and a half but not randomized

Hospital _____ Province _____

Patient's personal data:

Last name and name (initials)_____ ID _____

Date of birth (Day/Month/Year) ___/___/___ Sex (M/F) _____

Date of stroke onset (Day/Month/Year) ___/___/___

Reasons for exclusion:

- ☐ Unavailable interventional neuroradiologist
- ☐ Angiography room not available
- ☐ Impossible to transport to angiography room
- ☐ Patient refuses consent
- ☐ Family members refuse consent
- ☐ Non-functional randomization system
- ☐ Important disability before stroke
- ☐ Other (specify)_____

APPENDIX D: Data accessible by the doctor authorized to carry out the follow-up at 90 days

(to be completed by the physician that fills out the post-randomisation CRF)

Hospital _____ Province _____

Patient's personal data:

Last name and name (initials)_____ ID _____
Date of birth (Day/Month/Year) ___/___/___ Sex (M/F) _____

Patient's complete address at discharge:

_____
_____
Zip code _____
Tel (home and mobile) _____

Data of family doctor:
Name of family doctor _____
Address of family doctor:_____
Zip code_____Tel._____

Indicate the name of person to contact if necessary
Nome:_____
Relationship: _____
Address:_____
Zip code_____Tel._____

Form filled out by_____
on (Day/Month/Year) ___/___/___

APPENDIX E: CRF Glossary

CT SCAN

Infarct Hypodense areas due to recent ischemic lesion in accordance with neurological deficit.

Hemorrhagic infarct One or more hyperdensity areas due to presence of blood, with speckled or mottled appearance and with indistinct margins, in the context of area of low attenuation representing infarction or edema.

Intracerebral hemorrhage Very dense, homogeneous region of increased density with distinct margins with or without mass effect including all or the major part of the infarcted lesion.

Other hemorrhages Intraparenchimal hemorrhage not related to the previous infarct or subdural hematoma or subarachnoid hemorrhage.

ETHIOLOGIC DIAGNOSIS

Cardioembolism: the arterial occlusion is presumably due to an embolus arising in the heart when there is one of the following high-risk cardiac source of embolism: mechanical prosthetic valve, atrial fibrillation with or without valvular heart disease, rheumatic mitral stenosis, atrial appendage thrombus, dilated cardiomiopathy, atrial mixoma, recent myocardial infarction with anterior wall infarction and/or akinetic segment and/or intraventricular thrombus. Diagnostic studies should exclude dissection as a possible cause of stroke.

Dissection: angiographic appearance of elongated and tapering stenosis, possibly with complete occlusion of the lumen and/or signs of intimal flap, a pseudoaneurysm (i.e. an aneurismal bulging of the adventitial wall to the false lumen) or a double lumen.

Large -artery atherosclerosis: angiographic findings of >50% stenosis or occlusion of a major brain artery or branch cortical artery, presumably due to atherosclerosis. A history of intermittent claudicatio, transient ischemic attacks (TIAs) in the same vascular territory, diminished pulses helps support the clinical diagnosis.

Diagnostic studies should exclude potential high-risk sources of cardiogenic embolism, dissection and other causes of stroke.

Disease of small vessels: evidence at control CT scan of subcortical infarcts ≤ 1.5cm in diameter or normal CT scan and a reasonable syndrome gap (motor stroke and/or pure sensory, hemiparesis, ataxia, in absence of disorders of the visual field, or a deficit due to higher nervous functions of new occurrence, or alterations in the brainstem, at the time of worst neurological deficit).

Other causes: diagnostic studies identify other ethiology such as: non-atherosclerotic vasculopaties, hypercoagulability states and hematological disorders. Diagnostic studies should exclude cardioembolism and dissection as possible cause of stroke.

Unknown causes: after excluding atherosclerotic, high-risk cardiac sources, dissection and other causes of thrombo-embolism.

Existing events during hospitalization and cause of death

Symptomatic intracranial hemorrhage Sudden neurological worsening after a period of stable condition or recovery, with documented intracranial hemorrhage (CT scan or autopsy). Neurological worsening is defined by one or more of the following:

1. any major change in the level of consciousness
2. any substantial change in degree of motor deficit
3. new deficits that are clinically significant and persistent

Extracranial bleeding It is classified as mild if bleeding did not required blood replacement, or as severe if requiring blood replacement.

New ichemic stroke Sudden neurological worsening (see intracranial hemorrhage) after a period of recovery or stable condition without documented intracranial hemorrhage or cerebral edema from the previous ischemic lesion.

Cerebral edema Neurological worsening, as described above, after a period of stable conditions or improvements in clinical conditions, due to the development of significant mass effect of the recent lesion, with midline shift.

Myocardial infarction At least two of the following: typical history, new appearance of abnormal Q waves on EKG, peak enzymes levels exceeding (twice the upper limit of normal).
Pulmonary thromboembolism Sudden appearance of dyspnea with or without chest pain. Suspect diagnosis must be confirmed by lung CT with contrast medium or autopsy.
Pulmonary edema Sudden appearance of dyspnea with aspiratory wheezing, in all lung fields, tachycardia, high blood pressure, urine contraction and chest x-ray compatible with lung congestion.
Anaphylactic shock Sudden respiratory distress with urticaria or angioedema followed by arterial hypotension (SBP < 90 mmHg) and oliguria (< 20 ml/hr) persisting for more than one hour, and within 12 hours of treatment.

APPENDIX F: Management during the first 7 days

Components of care after admission into the Hospital

- Bed rest progressing to full activity as tolerated:
- Care of bedridden patients
- Skin and joint care
- Bronchopulmonary care
- Watch for neurological worsening or hypotension during mobilization
- Measure vital and neurological signs:
- Neurological worsening
- Fever
- Hypertension or hypotension
- Cardiac monitoring during first 24 hours
- Assess swallowing before starting oral intake of fluids or solids; advance diet as tolerated
- Intravenous fluids to avoid dehydration
- Nasogastric tube feedings for patients who can not swallow
- Avoid indwelling bladder catheter if possible
- Symptomatic treatment of pain, nausea, agitation
- Treat medical or neurological complications
- Treat hearth disease and other co-morbid diseases
- Prophylaxis against deep-vein thrombosis

Acute treatment of hypertension

- Treat anxiety, pain, nausea, vomiting
- Treat increased intracranial pressure
- Do not acutely treat an elevated blood pressure
- If possible, give oral agents or reinstitute medications given before the stroke
- Gradually lower the blood pressure
- Monitor blood pressure at least every 30 minutes for two hours:

1. If systolic blood pressure is > 180 mmHg and/or diastolic blood pressure is 105 to 140 mmHg for two or more readings 5 to 10 minutes apart:

- Give intravenous Labetalol, 10 mg over 1 to 2 minutes in bolus.
- Monitor blood pressure every 15 minutes during Labetalol treatment and observe for development of hypotension.
- The dose of Labetalol may be repeated or doubled every 10 to 20 minutes up a total dose of 150 mg.

2. If diastolic blood pressure is > 140 mmHg for two separate readings 5 to 10 minutes apart or if the preceding treatment did not give satisfactory response:

- Infuse sodium Nitroprusside (0.5 to 10 mg/Kg/min).
- Monitor blood pressure every 15 minutes during infusion of sodium Nitroprusside and observe for development of hypotension.

If systolic blood pressure is <180 and/or diastolic blood pressure is <105 anti-hypertension treatment is usually discouraged. Conversely, hemorrhagic transformation requires a treatment of high blood pressure more aggressive than that outlined above because of the risk of continued bleeding or recurrent hemorrhage

Acute anticoagulant therapy

- Possible indications: high-risk source of embolism (e.g. mechanical prosthetic valve), pulmonary embolism, and "overt" deep vein thrombosis.
- Do not treat with full-dose oral anticoagulants or unfractionated heparin (e.g. to PT, INR > 1.5 with oral anticoagulant; to aPTT >1.2 fold normal with unfractionated heparin) if patient presents with ischemic lesion detectable by CT scan that is > 33% of the MCA territory, or any type of intracranial hemorrhage, unless the patient has a life-threatening condition (e.g. pulmonary embolus).

• Patients at high risk of deep venous thrombosis (e.g. plegia, obesity and obligated bed rest) should be placed on low dose unfractionated heparin (5000 units subcutaneous every 8 or 12 hours) or low molecular heparin (preferably) at prophylactic doses (4000 units subcutaneous). Otherwise physical prevention (e.g. pressure stockings and mobilization) is recommended.
• Intravenous unfractionated heparin should be initiated with weight based bolus infusion, and adjusted according to the weight-based nomogram until a therapeutic level (according to the aPTT) is reached.
• Acute anticoagulant treatment with unfractionated heparin usually requires an initial bolus injection of 5000-10,000 units followed by a continuous infusion of about 900 units/h or 10-15 units/Kg/h to maintain the PTT at 2-2.50 times that of the control time.

Treatment of increased intracranial pressure (brain edema, mass effect, hydrocephalus)
General prophylaxis
• Control fever, agitation, nausea and vomiting, hypoxia, hypercapnia
• Modest fluid restriction (approximately 1.5 L to 2 L/day)
• Avoid potential hypo-osmolar IV fluids
• Elevate the head of the bed to augment venous drainage (30°)
Acute interventions
• Mannitol 0.5 g/Kg given in a 18-20% solution over 20 to 30 minutes
• Can repeat 0.25 g/Kg every 6 hours as needed
• Usual maximal daily dose is 2g/Kg
• Replace lost fluids
• Furosemide 20 to 40 mg given IV

Treatment of intracranial hemorrhage following thrombolysis
• Stop any thrombolytic, anticoagulant or antiplatelet therapy
• Check hemoglobin, hematocrit, PT, aPTT, platelet count and fibrinogen
• Type and cross match 4 units of blood
• Give 4 to 6 units of cryoprecipitate to rise fibrinogen level to >150 mg/dl
• Recheck fibrinogen level every 4 hours and transfuse with cryoprecipitate to maintain fibrinogen level >150 mg/dl
• The hemostatic defect must be corrected before any surgery can be performed
• The blood pressure will need aggressive treatment because of the risk of continued bleeding or recurrent hemorrhage

APPENDIX G: Requirements of the participating centers

The participating centers must have a stroke unit and a department of interventional neuroradiology:
Stroke Unit: at least 4 beds dedicated to the care of stroke. Priority will be given to the centers that have participated to the SITS-MOST or that have experience in trials on thrombolysis.
Department of Interventional Neuroradiology: availability of working team in emergency, an anesthesiologist to assist the procedure if the patient requires sedation for treatment, interventional angiography operator, an angiography room nurse, a technician. The angiography operator must have experience in catheterisation of intracranial vessels and must have completed at least 10 endovascular treatment interventions (aneurysms, arteriovenous malformations, stent or thrombolysis).

SUMMARY OF PROTOCOL AMENDMENTS

Two amendments were proposed on 15th December 2008 and approved by the Independent Ethics Committee of the leading center on 16th January 2009:

1) the extension of the therapeutical window from 3 h to 4:30 h in order to randomize patients within 4:30 h from stroke onset, on the basis of the ECASS III study results (N Engl J Med 2008;359:1317-29);

2) the introduction of a Neuroradiology Committee composed of experts in the field neuroradiology, interventional neurovascular and cerebrovascular diseases, to re-evaluate the neuro-imaging of the randomized patients.

In the occasion of the two amendments, we changed the exclusion criteria “Patients treated with oral anticoagulants” into “Patients treated with oral anticoagulants and INR>1.6”.

As a consequence, there are only two versions of the protocol: the original “Version 1-August 2007” and the final, containing changes according to the amendments, “Version 2-December 2008”. Changes of the original protocol were highlighted in yellow in the enclosed “Version 2-December 2008” protocol.

The study protocol, all amendments, the patient's information sheet and the consent form have been approved by each of the centers' Independent Ethics Committee.