Clinical Study Protocol

Efficacy and safety of MRI-based thrombolysis in wake-up stroke: a randomised, double-blind, placebo-controlled trial

WAKE-UP

Study-No.:	WAKE-UP
EudraCT No.:	2011-005906-32
Sponsor:	Universitätsklinikum Hamburg-Eppendorf (UKE) Martinistr. 52 20246 Hamburg Germany
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1. Summary

Study Title	Efficacy and safety of MRI-based thrombolysis in wake-up stroke: a randomised, double-blind, placebo-controlled trial
Study-No.	WAKE-UP
EudraCT No.	2011-005906-32
Sponsor	University Medical Center Hamburg-Eppendorf
Study Centres	Approximately approximately 60 centres in 6 European countries
Number of Patients	800 (400 subjects per arm)
Study Duration	June 2012 (first patient in) until August 2016 (data base lock)
Study Objectives	To test efficacy and safety of MRI-based intravenous thrombolysis with Alteplase in patients waking up with stroke symptoms or patients with unknown symptom onset.
Study Design	Interventional, treatment, randomised, double-blind, placebo-controlled, parallel assignment, international, multi-centre trial
Study Population	Patients with acute ischemic stroke proven by MRI and unknown time from symptom onset which otherwise fulfil the approval criteria for intravenous thrombolysis in acute stroke
Inclusion Criteria	*Clinical Inclusion Criteria* • Clinical diagnosis of acute ischemic stroke with unknown symptom onset (e.g., stroke symptoms recognized on awakening) • Last known well (without neurological symptoms) >4.5 hours of treatment initiation • Measurable disabling neurological deficit (defined as an impairment of one or more of the following: language, motor function, cognition, gaze, vision, neglect) • Age 18-80 years • Treatment can be started within 4.5 hours of symptom recognition (e.g., awakening) • Written informed consent by patient or proxy • Applicable for France only: subjects covered by or having the right to social security *Imaging Inclusion Criteria:* • Acute stroke MRI including diffusion weighted imaging (DWI), fluid attenuated inversion recovery (FLAIR), and MR-angiography (MRA) completed and showing a pattern of “DWI-FLAIR-mismatch”, i.e. acute ischemic lesion visibly on DWI (“positive DWI”) but no marked parenchymal hyperintensity visible on FLAIR (“negative FLAIR”) indicative of an acute ischemic lesion ≤4.5 hours of age
Exclusion Criteria	*Clinical Exclusion Criteria* • Planned or anticipated treatment with endovascular reperfusion strategies (e.g. intra-arterial thrombolysis, mechanical recanalization techniques) • Pre-stroke disability (inability to carry out all daily activities, requiring some help or supervision, i.e. slight disability

corresponding to an MRS score >1)

- Participation in any investigational study in the previous 30 days
- Severe stroke by clinical assessment (e.g. NIHSS >25)
- Hypersensitivity to Alteplase or any of the excipients
- Pregnancy or lactating (formal testing needed in woman of childbearing potential; childbearing potential is assumed in women up to 55 years of age)
- Significant bleeding disorder at present or within past 6 months
- Known haemorrhagic diathesis
- Manifest or recent severe or dangerous bleeding
- Known history of or suspected intracranial haemorrhage
- Suspected subarachnoid haemorrhage (even if CT is negative) or condition after subarachnoid haemorrhage from aneurysm
- History of CNS damage (e.g. neoplasm, aneurysm, intracranial or spinal surgery)
- Recent (within 10 days) traumatic external heart massage, obstetrical delivery, recent puncture of a non-compressible blood-vessel
- Current effective use of anticoagulants (e.g. Phenprocoumon, Warfarin, or new direct oral anticoagulants such as Apixaban, Dabigatran or Rivaroxaban) or current use of heparin and elevated thromboplastin time (low-dose subcutaneous heparin is allowed); inclusion may be considered in patients using vitamin K-antagonists (Phenprocoumon or Warfarin) when appropriate tests of anticoagulant activity (INR) show no clinically relevant activity
- Platelet count <100.000/mm^3 (<100G/l)
- Blood glucose <50 or >400 mg/dl (<2.8 or 22.2 mmol/l)
- Severe uncontrolled hypertension, i.e. systolic blood pressure >185 mmHg or diastolic blood pressure >110 mmHg or requiring aggressive medication to maintain blood pressure within these limits (routine medical treatment is allowed to lower the blood pressure below these limits)
- Manifest or recent bacterial endocarditis, pericarditis
- Manifest or recent acute pancreatitis
- Documented ulcerative gastrointestinal disease during the last 3 months, oesophageal varices, arterial aneurysm, arterial/venous malformations
- Neoplasm with increased bleeding risk
- Manifest severe liver disease including hepatic failure, cirrhosis, portal hypertension and active hepatitis
- Major surgery or significant trauma in past 3 months
- Stroke within 30 days
- Life expectancy 6 months or less by judgement of the investigator
- Any condition associated with a significantly increased risk of

	severe bleeding not mentioned above • Any contraindication to MRI (e.g. cardiac pacemaker) *Imaging Exclusion Criteria:* • Poor MRI quality precluding interpretation according to the study protocol • Any sign of intracranial haemorrhage on baseline MRI • FLAIR showing a marked parenchymal hyperintensity in a region corresponding to the acute DWI lesion indicative of an acute ischemic lesion with a high likelihood of being > 4.5 hours old • Large DWI lesion volume > 1/3 of the MCA or >50% of the anterior cerebral artery (ACA) or posterior cerebral artery (PCA) territory (visual inspection) or >100 ml • Any MRI findings indicative of a high risk of symptomatic intracranial haemorrhage related to potential IV-tPA treatment in the judgement of the investigator Inclusion and exclusion criteria will be assessed based on information available during the screening period. There will be no retrospective exclusion of patients based on information coming to knowledge later on.
Study drug	Active drug: intravenous Tissue Plasminogen Activator (rtPA, Alteplase, Actilyse®), 0.9 mg/kg body-weight up to a maximum of 90 mg, 10% as bolus, 90% over 1 hour as infusion (supplied as provided by the manufacturer as lyophilised powder to be reconstituted as a solution) Placebo: lyophilised powder to be reconstituted as solution indistinguishable from the active drug
Randomization	Eligible patients are randomized to treatment with either Alteplase (rtPA, Actilyse®) or placebo with a ratio of 1:1. Randomization is stratified according to age (≤60/>60 years) and severity of symptoms (NIHSS ≤10/>10) in four cohorts.
Criteria for Evaluation – Efficacy	*Primary Efficacy Endpoint* • "Favourable outcome" defined by a score of 0-1 on the modified Rankin Scale (MRS) 90 (±10) days after stroke *Secondary Efficacy Endpoints* • Global Outcome Score (combination of MRS 0-1, NIHSS 0-1, Barthel Index [BI] 95-100, Glasgow Outcome Scale [GOS] 1) 90 (±10) days after stroke • Categorical shift in MRS 90 (±10) days after stroke • Responder analysis relating MRS 90 (±10) days after stroke to baseline NIHSS score: "response" defined by NIHSS <7 = MRS 0; NIHSS 8-14 = MRS 0-1; NIHSS >14 = MRS 0-2 • Infarct volume after 22-36 hours • Depressive symptoms 90 (±10) days after stroke (Beck Depression Inventory [BDI]) • Functional health status and quality of life 90 (±10) days after stroke (EQ-5D) • Use of health care system resources

Criteria for Evaluation – Safety	*Primary Safety Endpoints* • Mortality 90 (±10) days after stroke • Death or dependency 90 (±10) days after stroke (MRS 4-6) *Secondary Safety Endpoints* • Symptomatic intracranial haemorrhage (SICH) as defined in SITS-MOST • SICH as defined ECASS II • SICH as defined in NINDS • Parenchymal haemorrhage type 2 (PH-2)
Statistical Methods	All analyses will be conducted on data from all randomly assigned patients, whether or not treated, according to the intention-to-treat principle. All efforts will be made to minimize the amount of missing data. Sensitivity analyses based on different hypotheses about the missingness pattern of the primary outcome will be performed to test for the robustness of the primary analysis. Analyses will also be repeated according the per protocol principle. The judgements of central image reading will be used to define the population for per protocol analysis. One interim analysis of primary endpoint is planned after the inclusion of 500 patients (250 in each group), with a statistical stopping guideline for an overwhelming benefit. *Analysis of Efficacy – primary endpoint*: The primary efficacy endpoint is disability evaluated 90 (±10) days after stroke using the modified Rankin Scale (MRS), dichotomized in favourable (MRS 0-1) and unfavourable (MRS 2-6) outcome. One interim analysis of primary endpoint is planned after the inclusion of 500 patients (250 in each group), with a statistical stopping guideline for an overwhelming benefit. A Lan-DeMets alpha spending function will be used to control for the overall alpha level, using O'Brian and Fleming boundaries (corresponding to alpha-level of 0.0132 and 0.0460 at the interim and final analysis, respectively). Those values will be adapted depending on the effective number of patients analysed at the time of the analyses). Between-group differences will be tested using a chi-square test. An unconditional logistic regression model will be fitted to estimate the odds-ratio associated with treatment effect, restricting the adjustment for the randomisation stratified factors (age and symptoms severity). Corresponding confidence intervals will be provided. In a complementary analysis, a more complete model will be fitted, retaining also (both clinical and imaging) baseline variables with p-value <0.10. *Analysis of Efficacy – secondary endpoints*: Global Outcome Score analysis: a global odds-ratio test based on a linear logistic-regression model will be performed – a method that uses generalised estimation equations to perform a Wald-type test – to compare the proportion of favourable outcome in the Alteplase arm and the placebo arm. Responder analysis: treatment effect will be analysed using the odds-ratio estimate and its 95% confidence interval. Analysis of the categorical shift in MRS: The categorical shift in MRS 90 (±10) days after stroke will be analysed fitting a log-linear model for ordinal data. Infarct volume after 22-36 hours: infarct volume at follow-up after 22-36 hours will be measured by MRI and compared between the Alteplase arm and the placebo arm using a Student's t-test, the variable being transformed if necessary. A multivariate analysis will be performed

fitting a linear regression model.

Depressive symptoms 90 (±10) days after symptom onset (BDI): BDI values will be compared between the Alteplase arm and the placebo arm both for absolute values and for the proportion of patients reaching scoring within the predefined categories of minimal depression, mild depression, moderate depression, and severe depression. Absolute values will be compared between the Alteplase arm and the placebo arm using a Student's t-test, the variable being transformed if necessary. A multivariate analysis will be performed fitting a linear regression model. The distribution of the categorized scores between the two arms will be compared fitting polytomous ordinal logistic regression models.

Functional health status and quality of life 90 (±10) days after stroke (EQ-5D): The summary index (EQ-5D Index) and the proportion of patients with good quality of life according to a pre-specified cut-off (EQ-5D Index ≥70) will be compared between the Alteplase arm and the placebo arm using Student's t-test or a chi-square test as appropriate. An unconditional logistic regression model will be fitted to estimate the odds-ratio associated with treatment effect.

Use of health care system resources: the use of health care system resources will be described for both groups and comparison performed using linear or generalized linear models, according to the type of variables.

Analysis of Safety:

Mortality and death or dependency 90 (±10) days after stroke will be analysed sequentially after inclusion of 100, 200, 300, 500, and 800 patients. A Lan-DeMets alpha spending function will be used to control for the overall alpha level, using Hwang-Shih-DeCani boundaries with parameter 1.2. The stopping rule proposed to the DSMB is to consider stopping the trial after an interim analysis if the proportion of death, or the proportion of the combined endpoint death or dependency (defined by an MRS score of 4-6) in the Alteplase group exceeds the one in the placebo group with a Chi-square test value greater than the threshold defined by the alpha-spending function (one sided test, overall alpha level of 0.10 for each endpoint). These stopping rules will be considered as guidelines and will not be binding to the DSMB. The decision to stop or continue the trial will be based on the overall assessment of risk and benefit.

The occurrence of SICH (as defined in SITS-MOST, ECASS II, NINDS) and PH-2 will also be compared between the test and the control arm but will not be used as endpoints for formal stopping rules. Formally, these variables will not be considered as sequentially analyzed. All safety variables being binary, an unconditional logistic regression model will be fitted to estimate the odds-ratio associated with treatment effect.

Analysis of Baseline Symptom Severity:

In order to ensure the enrolment of a typical population of acute ischemic stroke patients with on average moderate to severe disabling neurological symptoms the severity of symptoms at baseline measured by the NIHSS will be analysed sequentially after inclusion of 100, 200, 500, and 800. Descriptive statistics (median, IQR, mean, SD) will be computed. In case of baseline symptom severity outside the expected range (e.g. a median NIHSS below or above the expected range of 9-11) the Steering Committee might consider a modification of the clinical inclusion criteria (e.g. specify a lower threshold on the NIHSS).

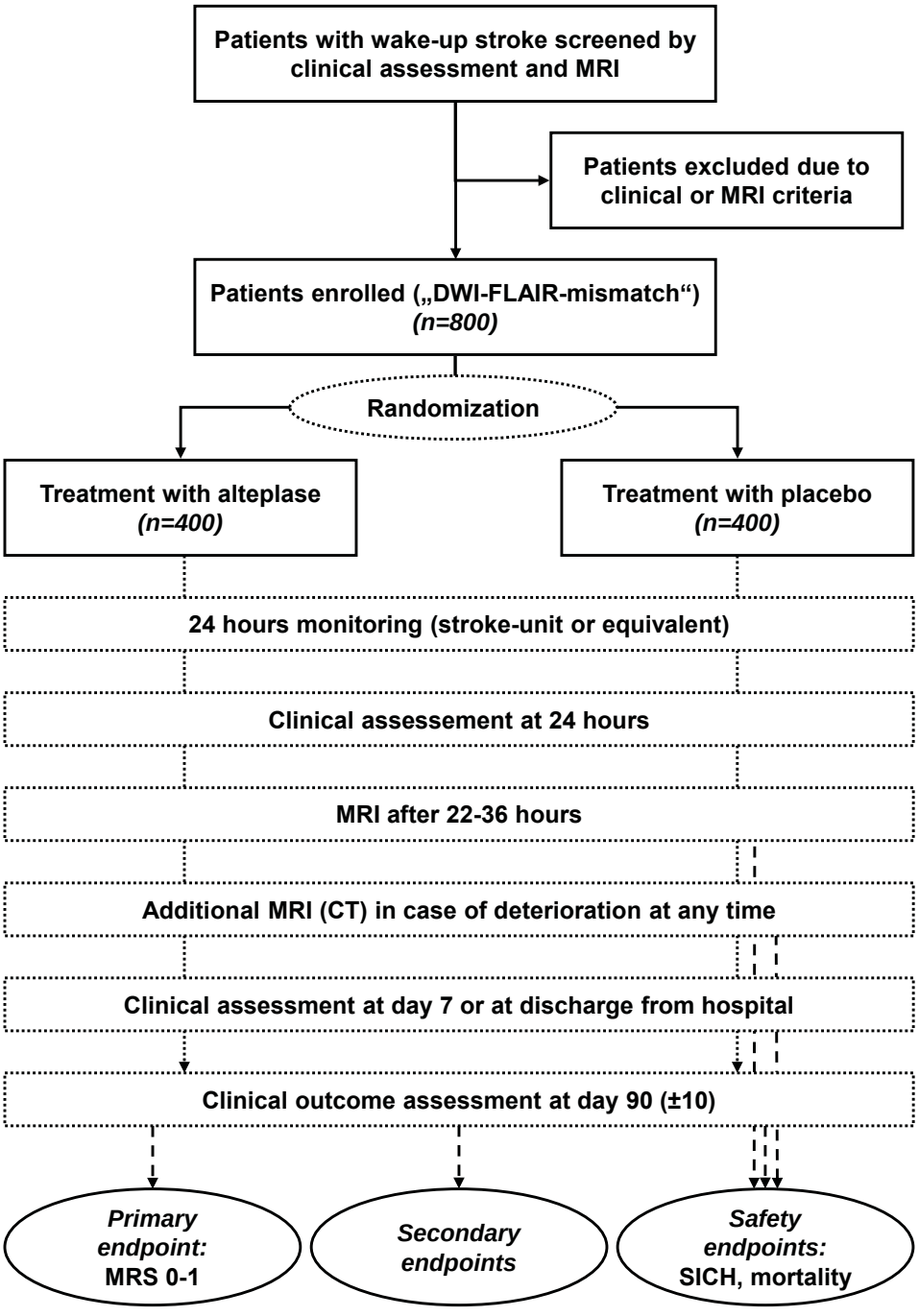
Sample Size Calculation

The sample size is based on the primary efficacy endpoint ("favourable outcome" 90 (±10) days after stroke as defined by a MRS score of 0-1). We expect a 10.0% absolute difference in the proportion of patients

	between patients treated with the active drug and placebo (expected rate of “favourable outcome” 43.3% in patients treated with Alteplase and 33.3% in patients in the placebo group). In order to demonstrate the expected treatment effect (i.e. to reject the null hypothesis of an identical proportion of favourable outcome in the two groups) with a type-1 error alpha = 0.05 (two-tailed) a power of 80% (beta = 20%) is achieved with a sample size of n=740 patients (n=370 per treatment group). Accounting for possible treatment failures, protocol violations and drop-outs, we plan to enrol n=800 patients (n=400 per treatment group).
Concomitant Treatment or Medication	The following treatment or medication is not allowed together with and for 24 hours after treatment with the study drug: • Any thrombolytic treatment • Any endovascular or mechanical recanalization procedure • Any antiplatelet agent • Any anticoagulant

2. Flow Chart and Schedule of Assessments

2.1. Flow Chart



2.2. Schedule of Assessments

	Visit (Time in hours / days from treatment = V1)						
	Screening		*Treatment*			*Follow Up*	
	V0	R	V1	V2[a] (1-24h)	V3 (22-36h)	V4[b] (7±2d)	V5 (90±10d)
Informed consent	X						
Demographic data	X						
Medical history	X						
Physical examination	X				X	X	X
Pre-stroke MRS	X						
NIHSS	X				X	X	X
MRS							X
BI							X
GOS							X
BDI							X
EQ-5D							X
Questionnaire: use of health care system resources							X
Previous medication	X						
Blood pressure and heart rate	X		X	X[e]	X	X	
Body temperature	X			X[f]	X	X	
Local laboratory: INR	X				X		
Local laboratory: haematology, serum glucose	X				X		
Pregnancy test[c]	X						
Brain MRI scan[d]	X				X		
Inclusion / exclusion criteria	X						
Randomization		X					
Study medication (bolus + 1h infusion)			X				
Concomitant medication	X		X	X	X	X	X
12-lead ECG	X						
24h monitoring				X[a]			
Adverse events	X		X	X	X	X	X

V = Visit; R = Randomization; h = hour; d = day

[a] 24h monitoring in Stroke Unit or equivalent unit including repeated measurements of blood pressure, heart rate and body temperature

[b] Or hospital discharge if <5 days

[c] Mandatory for women of childbearing potential

[d] Also to be performed in any case of neurological deterioration during the first 72h

[e] every hour

[f] every six hours

3. List of Abbreviations

ADC	Apparent diffusion coefficient
AE	Adverse event
AUH	Aarhus University Hospital
BDI	Beck Depression Inventory
BI	Barthel Index
CFIN	Center of Functionally Integrative Neuroscience
Charité	Charité – Universitätsmedizin Berlin
CRO	Contract Research Organisation
CSB	Center for Stroke Research Berlin
CSF	Cerebrospinal fluid
CT	Computed tomography
CIRB	Central Image Reading Board
DALY	Disability adjusted life years
DEFUSE	Diffusion-weighted Imaging Evaluation For Understanding Stroke Evolution
DIAS	Desmoteplase in Acute Stroke trial
DSMB	Data and Safety Monitoring Board
DWI	Diffusion weighted imaging
DWI-FLAIR-mismatch	Mismatch between a visible lesion on DWI and a normal FLAIR image
EAB	Ethics Advisory Board
ECASS	European Cooperative Acute Stroke Study
EC	European Commission
ECASS II	Second European-Australasian Cooperative Acute Stroke Study
ECASS III	Third European Cooperative Acute Stroke Study
EC	European Commission
ECG	Electrocardiogram
EPITHET	Echoplanar Imaging Thrombolysis Evaluation Trial
EQ-5D	Standardised instrument for use as a measure of health outcome (quality of life)
EU	European Union
EXTEND	Extending the Time for Thrombolysis in Emergency Neurological Deficits trial
FP7	Seventh Framework Programme
FLAIR	Fluid attenuated inversion recovery
GOS	Glagow outcome scale
HCL	Hospices Civils de Lyon
ICH	Intracranial haemorrhage
IDIBGi	Institut d'Investgació Biomèdica de Girona
INR	International Normalized Ratio
KUL	Katholieke Universiteit Leuven
MRI	Magnetic resonance imaging
MRS	Modified Rankin scale
MR-WITNESS	Multi-Center Safety Trial of IV rt-PA in Patients With Unwitnessed Stroke Onset
NHS	National Health Service

NIH	National Institutes of Health
NIHSS	National Institutes of Health Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
NPV	Negative predictive value
PH	Parenchymal haemorrhage
PI	Perfusion imaging
PPV	Positive predictive value
PRE-FLAIR	PREdictive value of FLAIR and DWI for the identification of acute ischemic stroke patients ≤ 3 and ≤ 4.5 h of symptom onset – a multicenter study
rtPA	Recombinant tissue plasminogen activator
SAB	Scientific Advisory Board
SAE	Serious adverse event
SAFE	Stroke Alliance for Europe
SICH	Symptomatic intracranial haemorrhage
SME	Small and medium sized enterprise
SITS-MOST	Safe Implementation of Thrombolysis in Stroke Monitoring Study
SITS-ISTR	Safe Implementation of Thrombolysis in Stroke International Stroke Thrombolysis Register
SC	Steering Committee
STIR	Stroke Imaging Repository
SUSAR	Suspected Unexpected Adverse Reaction
T2wI	T2-weighted imaging
UG	University of Glasgow
UKE	University Medical Center Hamburg-Eppendorf
VISTA	Virtual International Stroke Trials Archive
WHO	World Health Organization

4. Background

4.1. Stroke and Thrombolysis

The burden of stroke

Stroke is the 2nd most common single cause of death and the most frequent cause of permanent disability in industrialised countries. The WHO estimates that 15 million people suffer from stroke each year and 5 million are left permanently disabled (WHO, 2004). In the EU stroke accounts for just over 500.000 deaths each year with just around one in ten men (9%) and one in eight women (12%) dying from stroke (Allender et al., 2008).

Thrombolysis in acute ischemic stroke: current status and the role of the time-window

Intravenous thrombolysis with recombinant tissue Plasminogen Activator (rtPA, Alteplase) represents the only effective and approved specific treatment for acute ischemic stroke and is recommended by international, European and national guidelines (ESO, 2008). Following

the first positive clinical trial of thrombolysis in ischemic stroke, treatment with Alteplase was restricted to treatment initiation ≤3 hours of symptom onset. Given the recent proof of an effect up to 4.5 hours in ECASS III (Hacke et al., 2008), international guidelines have already modified the recommendations for thrombolysis (Del Zoppo et al., 2009), and the European Medicines Agency has recently approved intravenous thrombolysis with Alteplase for acute stroke up to 4.5 hours of symptom onset. Anyway, information on the time point of symptom onset is a prerequisite for treatment with intravenous thrombolysis, and an unknown time window represents a contraindication against thrombolysis in stroke.

Stroke imaging: current status, ongoing debates

In regards to imaging, non-contrast computed tomography (CT) was the modality used in the initial clinical trials of thrombolysis in stroke. Exclusion of intracranial haemorrhage is the only requirement concerning brain imaging demanded by the approval criteria for thrombolysis. There is, however, an ongoing scientific debate regarding the potential advantages of using modern multiparametric imaging to guide acute stroke treatment. Research activities and discussion have mainly focussed on the role of multiparametric MRI, so called “stroke MRI”, including diffusion weighted imaging (DWI), perfusion imaging (PI), and magnetic resonance (MR) angiography. Stroke MRI has brought about a revolution to stroke imaging (Muir et al., 2006). DWI allows for identification of ischemic tissue within minutes of onset (Moseley et al., 1990), while PI detects critically hypoperfused brain tissue (Ostergaard et al., 1996). Referencing to experimental data and the pathophysiological concept of an ischemic penumbra, a mismatch between a large area of hypoperfusion and a smaller diffusion lesion has been hypothesized to represent potentially salvageable tissue at risk of infarction and might be used to identify patients likely to benefit from reperfusion treatment even beyond a strict time window. However, randomised controlled trials have failed to prove efficacy of penumbral imaging based thrombolysis in an extended time window as yet (Davis et al., 2008; Furlan et al., 2006; Hacke et al., 2005; Hacke et al., 2009).

4.2. Wake-up Stroke

The challenge of wake-up stroke

Up to 20% of strokes are estimated to occur during sleep, adding up to an estimated 3 million strokes per year world-wide. There are even observations pointing towards strokes occurring during sleep being more severe (Jimenez-Conde et al., 2007). In another study, patients with stroke on awakening were less likely to return home than patients with known symptom onset (Nadeau et al., 2005). Given approval criteria and guideline recommendations as outlined above, this large group of patients is a priori excluded from thrombolysis. This is an unsatisfactory situation and has led to considerations of alternative approaches. Carefully performed clinical and imaging observations suggest that in a large number of patients

waking up with stroke symptoms strokes may have occurred in the early morning hours so that they might still be eligible for thrombolysis. There is a circadian clustering of stroke in the morning hours (Chaturvedi et al., 1999; Elliott et al., 1998) – an observation that was made before in myocardial infarction (Cannon et al., 1997; Muller et al., 1985). And there is growing evidence that patients studied within the first hours after waking up with stroke symptoms are in large parts similar as regards clinical and imaging findings to patients studied within the first hours of observed symptom onset. Comparable to imaging results of patients within up to 3 hours of known symptom onset 11% of patients who woke up with symptoms of stroke showed early ischemic signs on CT within 3 hours of symptom recognition (Todo et al., 2006). In another sample the frequency of early ischemic signs on CT was also comparable between patients with wake-up stroke (52%) and patients with stroke while awake (47%) in patients studied within 6 hours of stroke symptom awareness (Serena et al., 2003). The proportion of patients showing a perfusion-diffusion-mismatch pattern on MRI was also similar for wake-up strokes and patients <3 hours of symptom onset (Fink et al., 2002), a finding which was just recently reproduced for the detection of tissue at risk by perfusion CT in another large case series (Silva et al., 2010). Together these data suggest that a large number of patients with wake-up stroke might still be within a time window for thrombolysis when reaching the hospital.

Current clinical practice in wake-up stroke

There is no evidence for any specific acute treatment including thrombolysis in wake-up stroke from randomised controlled trials. Thrombolysis is not approved for acute stroke patients with unknown symptom onset. National, European, and international guidelines do not recommend thrombolysis in patients with unknown time of symptom onset including patients waking up with stroke symptoms unless the they have been witnessed to be without stroke symptoms less than 4.5 hours prior to treatment which is highly unlikely in case of waking up with stroke symptoms in the early morning hours (ESO, 2008; Del Zoppo et al., 2009). Current clinical practice of treatment of patients with wake-up stroke comprises CT to rule out intracranial haemorrhage or to diagnose ischemic infarction, monitoring of physiologic parameters on a specialized stroke unit, and early secondary prevention depending on the assumed stroke aetiology.

Imaging surrogate markers of lesion age

MRI findings change during the time course of acute cerebral ischemia, and it has been suggested to use the combination of different MRI sequences as a surrogate marker for the age of an acute ischemic lesion. Tissue water changes after ischemic stroke follow a characteristic course: the drop of the cerebral blood flow below a critical threshold leads to a disruption of the energy metabolism, resulting in cytotoxic edema which can be depicted by a reduced apparent diffusion coefficient (ADC) on DWI within minutes of stroke (Hoehn-

Berlage et al., 1995; Mintorovitch et al., 1991; Moseley et al., 1990). During the following 1-4 hours tissue osmolality increases, accompanied by a net increase of water (Schuier and Hossmann, 1980; Watanabe et al., 1977), which precedes the classic vasogenic edema (Go, 1997). This absolute increase of water content can be detected by T2-weighted MRI (Hoehn-Berlage et al., 1995; Kato et al., 1985; Venkatesan et al., 2000). Thus, DWI allows for the detection of acute ischemic lesions within minutes with a high contrast (Moseley et al., 1990), but does not allow any further conclusions on lesion age during the first hours of stroke, while T2 signal changes might allow further timely allocation of ischemic lesions. However, the identification of ischemic lesions on T2-weighted imaging (T2wI) is hampered by the high signal intensity of cerebrospinal fluid (CSF) with partial volume effects of CSF in particular limiting the detection of cortical lesions. Fluid attenuated inversion recovery (FLAIR) imaging with suppression of CSF signal and strong T2 weighting proved superior to T2wI in the detection of ischemic lesions (Brant-Zawadzki et al., 1996; Noguchi et al., 1997). There are a few studies on the performance of FLAIR sequences in the diagnosis of acute stroke which demonstrated its sensitivity to detect ischemic lesions after several hours of stroke onset (Gauvrit et al., 2006; Perkins et al., 2001; Ricci et al., 1999). The pattern of a visible ischemic lesion on DWI together with normal T2wI or FLAIR is a typical finding in human stroke if imaging is performed within the first hours of stroke (Lutsep et al., 1997; Schlaug et al., 1997; Sorensen et al., 1996). These results are also well in line with data from experimental stroke, where T2wI failed to detect acute ischemia until about 2-3 hours of stroke (Horikawa et al., 1986; Levy et al., 1983; Mintorovitch et al., 1991).

Preparatory work and a novel approach: DWI-FLAIR-mismatch

These observations have led to a new concept, the DWI-FLAIR-mismatch, introduced by the WAKE-UP coordinators (Thomalla et al., 2009) to identify patients likely to benefit from thrombolysis based on the assumed lesion age. In contrast to the previously suggested concept of perfusion-diffusion-mismatch, which labels a mismatch between lesion volumes on two parameter maps, DWI-FLAIR-mismatch refers to the mismatch between visibility of an ischemic lesion in one sequence (DWI), indicating the presence of acute ischemia, while it is not visible in the other sequence (FLAIR), indicating that the ischemic lesion is less than 3-4.5 hours old. Moreover, in contrast to perfusion-diffusion-mismatch, which indicates tissue viability, DWI-FLAIR-mismatch indicates lesion age, which is the essential piece of information missing in wake-up stroke.

In a preparatory study, partners of the WAKE-UP consortium were able to show that a mismatch between a visible lesion on DWI and a normal FLAIR reliably identifies patients within a time window of ≤3 hours with high specificity (0.93) and positive predictive value (0.98) (Thomalla et al., 2009). Moreover, a clear time-dependency of the visibility of acute ischemic lesions on FLAIR was demonstrated increasing to almost 100% after 3 hours.

These findings were confirmed in consecutive studies within the WAKE-UP consortium (Ebinger et al., 2010a; Ebinger et al., 2010b) and by other groups (Aoki et al., 2010). Following the results from the single centre pilot study, the WAKE-UP coordinators initiated and conducted a large prospective multicentre study which was published recently including n=643 patients (PRE-FLAIR: PREdictive value of FLAIR and DWI for the identification of acute ischemic stroke patients ≤ 3 and ≤ 4.5 h of symptom onset – a multicenter study) (Thomalla et al., 2011). This study reproduced the main findings of the previous single centre studies demonstrating 1) a clear time dependency of the visibility of acute ischemic lesions on FLAIR, and 2) high predictive values for the identification of patients with symptom onset <4.5 hours. Restricting the analysis to the assumed target population for thrombolysis as recently suggested (Aoki et al., 2010; Thomalla and Gerloff, 2010), i.e. n=408 patients with MCA stroke and relevant neurological deficit defined by a National Institutes of Health Stroke Scale (NIHSS) score >3, identified patients <4.5 hours with a specificity of 0.81 and a positive predictive value (PPV) of 0.87 (Thomalla et al., 2011). This is similar to recent results of a Japanese group reporting a specificity of 0.83 and a positive predictive value of 0.90 to identify patients with symptom onset <4.5 hours from n=214 patients with supra-tentorial non-lacunar ischemic stroke (Aoki et al., 2010). Together these studies suggest that the DWI-FLAIR-mismatch allows identifying patients with wake-up stroke with a sufficiently high likelihood of being in a time window in which thrombolysis is proven effective and safe (≤4.5 hours). WAKE-UP will be the first clinical trial to use the novel approach of DWI-FLAIR-mismatch to prospectively identify patients for thrombolysis. This will represent a paradigm change and a scientific breakthrough towards an imaging-guided individually tailored acute stroke treatment. If the trial will be positive, it will be the first positive clinical trial applying MRI criteria to select patients for thrombolysis in stroke.

Improving outcome in wake-up stroke by thrombolysis

Thrombolysis is a very effective treatment leading to an absolute increase of patients with favourable outcome ranging from 6.9% to 12.5% for patients treated within 4.5 hours depending on time from symptom onset to treatment (Lees et al., 2010) and an improved outcome across all disability ranges in estimated one third of patients treated (Saver, 2004). As patients with wake-up stroke were found to be comparable to patients with witnessed stroke onset we are confident that these treatment effects can be transferred to wake-up stroke patients. Thus, approximately one third of patients with wake-up stroke selected by the suggested imaging criteria are expected to benefit from thrombolysis with improved outcome across all ranges and with about 10% absolute increase of patients with a favourable outcome, i.e., no or only minimal neurological deficit three months after stroke.

Potential risks of thrombolysis in wake-up stroke: symptomatic intracranial haemorrhage

The only randomised controlled trial of acute treatment in wake-up stroke until now (AbBEST-II) was prematurely stopped due to an increase of symptomatic intracranial haemorrhage (SICH) (Adams et al., 2008a; Adams et al., 2008b). This trial used the Glycoprotein IIb/IIIa Receptor Antagonist Abciximab within 3 hours of awakening with stroke symptoms. The reasons for an increase of SICH in this trial, however, are manifold including a different drug and different imaging inclusion criteria, thus they must not be translated to MRI-thrombolysis with Alteplase in wake-up stroke. We are confident that we will not face an increased risk of SICH in our trial. The pattern of DWI-FLAIR-mismatch criterion assures a very high likelihood of enrolled patients being within a time window of 4.5 hours of symptom onset. The likelihood of ischemic lesions being less than 6 hours old is even higher (with a PPV of 0.95 in PRE-FLAIR and 0.98 in the Japanese sample (Aoki et al., 2010) in comparable patients). From the pooled analysis of the previous stroke trials and Cochrane analysis we know that thrombolysis can be performed within 6 hours of symptom onset without excess of intracranial haemorrhages (Lees et al., 2010; Wardlaw et al., 2009). Low rates of SICH in uncontrolled case series of thrombolysis in wake-up stroke further support this assumption (Barreto et al., 2009; Breuer et al., 2010; Cho et al., 2008). Moreover, to further ensure safety of patients enrolled and to reduce the potential risk of SICH we will exclude patients with very large DWI lesion volumes, which have been demonstrated to be at markedly higher risk of SICH (Singer et al., 2008).

MRI for acute stroke diagnostic: an increasing trend

During the past decade stroke MRI has become increasingly used for both scientific and clinical applications in the field of acute stroke (Muir et al., 2006). In a growing number of stroke centres MRI is used as primary diagnostic tool in day to day practice (Hjort et al., 2005). The increased scientific and clinical use of MRI in acute stroke is accompanied by an increased availability of MRI scanners in European countries during the past two decades. While in 1990 the average rate of MR units per million inhabitants was 1.7 in the EU, this rate has increased by more than five times to 9.8 (OECD, 2003; OECD, 2010). The increased availability and use of MRI in acute stroke will ensure the feasibility of an MRI based clinical trial such as WAKE-UP.

Wake-up stroke in other clinical trials

Currently no large randomised controlled trial targets patients waking up with stroke symptoms. The steering committee of the clinical trials of Desmoteplase in acute ischemic stroke (DIAS-3/4) has recently decided not to include wake-up stroke in the DIAS-3/4 trials (personal communication). The EXTEND trial is testing thrombolysis in an extended time window in patients with perfusion-diffusion-mismatch (ACTR Number:

ACTRN12610000011088; http://www.anzctr.org.au/ACTRN12610000011088.aspx). EXTEND allows for the inclusion of patients with wake-up stroke if treatment can be initiated within 9 hours of the assumed onset of stroke, which is calculated as the mean between last seen normal and waking up with stroke symptoms. Imaging inclusion criteria in EXTEND, however, are completely different from the approach used in WAKE-UP (using a “penumbral” pattern on stroke MRI defined by perfusion-diffusion-mismatch indicating tissue viability, instead of DWI-FLAIR-mismatch used in WAKE-UP to identify patients with stroke lesions ≤4.5 hours of age). Moreover, with an estimated sample size of 400 patients EXTEND will not be powered to prove efficacy in the likely rather small subgroup of patients with wake-up stroke (with estimated 80 patients, corresponding to 20% of the sample). There is also an uncontrolled safety trial of MRI based thrombolysis in preparation to be sponsored by the NIH (MR-WITNESS; ClinicalTrials.gov Identifier: NCT01282242; http://clinicaltrialsfeeds.org/clinical-trials/show/ NCT01282242). MR-WITNESS will use the DWI-FLAIR-mismatch concept amended by FLAIR signal intensity measurement to select an estimated of 80 patients for intravenous thrombolysis, in order to demonstrate safety of this approach. MR-WITNESS will also not be powered to prove efficacy in patients with wake-up stroke.

5. Objectives and Rationale

The purpose of WAKE-UP is to test efficacy and safety of MRI-based intravenous thrombolysis in patients waking up with stroke symptoms or patients with unknown symptom onset. The explanations above have described both the urgent need for a clinical trial of thrombolysis in wake-up stroke, and the scientific and clinical environment ensuring the feasibility of such a trial based on MRI. Currently, there is no specific acute treatment available for patients with wake-up stroke or otherwise unknown symptom onset. Approval criteria, guidelines and current clinical practice exclude these patients from thrombolysis. Only a randomised controlled trial will provide the proof of efficacy and safety of thrombolysis in wake-up. The use of MRI and the pattern of DWI-FLAIR-mismatch to select patients which was evaluated in a series of preparatory studies will ensure the enrolment of a sample of patients likely to benefit from thrombolysis. The increasing availability of MRI scanners and the increasing use of MRI in acute stroke in a growing number of centres will ensure the feasibility of an MRI based clinical trial of stroke thrombolysis. The findings of WAKE-UP are likely to have a direct impact on clinical practice. In case of a positive result this will provide evidence for an effective and safe treatment for a new large group of acute stroke patients currently excluded from specific acute treatment. Thus, WAKE-UP will improve treatment options for acute stroke patients and reduce the overall burden of stroke.

6. Alteplase (rtPA) in Acute Stroke

If not stated otherwise chapter 6 refers to the Summary of Product Characteristics for Actilyse® provided by the manufacturer of Actilyse®, Boehringer Ingelheim (Fachinformation Actilyse®, 2014).

6.1. Description of the Investigational Medicinal Product (IMP)

Alteplase is a tissue plasminogen activator produced by recombinant DNA technology (recombinant tissue-type plasminogen activator, rt-PA). It is a glycoprotein of 527 amino acids. Alteplase is a fibrinolytic substance manufactured by Boeringer Ingelheim and distributed in Europe under the registered trade name of Actilyse®.

Alteplase is an enzyme (serine protease) which has the property of fibrin-enhanced conversion of plasminogen to plasmin. It produces limited conversion of plasminogen in the absence of fibrin. When introduced into the systemic circulation at pharmacologic concentration, Alteplase binds to fibrin in a thrombus and converts the entrapped plasminogen to plasmin. This initiates local fibrinolysis with limited systemic proteolysis. Alteplase is rapidly cleared from the plasma with an initial half-life of less than 5 minutes. The plasma clearance of Alteplase is 380–570 mL/min. The clearance is mediated primarily by the liver. The initial volume of distribution approximates plasma volume.

Alteplase is a sterile, white to off-white, lyophilized powder for intravenous administration after reconstitution with Sterile Water for Injection.

6.2. Indications and Usage

Alteplase is approved for use in acute myocardial infarction, massive pulmonary embolism, and acute ischemic stroke.

6.3. Application

Treatment with Alteplase should only be initiated within 4.5 hours after the onset of stroke symptoms, and after exclusion of intracranial haemorrhage. Treatment with Alteplase should be initiated as early as possible after stroke symptom onset. The recommended and approved dose for treatment of acute ischemic stroke is 0.9 mg/kg (not to exceed 90 mg total dose) infused over 60 minutes with 10% of the total dose administered as an initial intravenous bolus over 1 minute.

6.4. Contraindications

Alteplase therapy in patients with acute ischemic stroke is contraindicated in the following situations, because of an increased risk of bleeding which could result in significant disability or death:

General contraindications

- Known haemorrhagic diathesis
- Patients currently receiving effective oral anticoagulants
- Manifest or recent severe or dangerous bleeding
- Known history of or suspected intracranial haemorrhage
- Suspected subarachnoid haemorrhage or condition after subarachnoid haemorrhage from aneurysm
- Any history of central nervous system (CNS) damage (e.g. neoplasm, aneurysm, intracranial or spinal surgery)
- Haemorrhagic retinopathy, e.g. in diabetes
- Recent (within 10 days) traumatic external heart massage, obstetrical delivery, recent puncture of a non-compressible blood-vessel
- Severe uncontrolled arterial hypertension
- Bacterial endocarditis, pericarditis
- Acute pancreatitis
- Documented ulcerative gastrointestinal disease during the last 3 months, oesophageal varices, arterial aneurysm, arterial/venous malformations
- Neoplasm with increased bleeding risk
- Severe liver disease including hepatic failure, cirrhosis, portal hypertension and active hepatitis
- Major surgery or significant trauma in past 3 months

Additional contraindications in acute ischemic stroke

- Symptoms of ischemic attack began more than 4.5 hours prior to infusion start or when time of symptom onset is unknown
- Minor neurological deficit or symptoms rapidly improving before start of infusion
- Severe stroke as assessed clinically and/or by appropriate imaging techniques

- Seizure at onset of stroke
- Evidence of intracranial haemorrhage on pretreatment evaluation
- Symptoms suggestive of subarachnoid hemorrhage even if CT scan is normal
- Administration of heparin within the previous 48 hours and a thromboplastin time exceeding the upper limit of normal for laboratory
- Patients with history of prior stroke and diabetes
- Prior stroke within the last 3 months
- Platelet count <100.000/mm^3 (<100G/l)
- Systolic blood pressure >185 mmHg or diastolic blood pressure >110 mmHg or management (ICV medication) necessary to reduce BP to these limits
- Blood glucose <50 or >400 mg/dl (<2.8 or 22.2 mmol/l)

6.5. Side Effects

The most common complication encountered during Alteplase therapy is bleeding. Allergic-type reactions, e.g., anaphylactoid reaction, laryngeal edema, orolingual angioedema, rash, and urticaria have been reported. A cause and effect relationship to Alteplase therapy has not been established. When such reactions occur, they usually respond to conventional therapy. There have been post-marketing reports of orolingual angioedema associated with the use of Alteplase.

In addition, the following adverse reactions have been reported among acute stroke patients receiving Alteplase in clinical trials and in post-marketing experience (these reactions are frequent sequelae of the underlying disease and the effect of Alteplase on the incidence of these events is unknown): Cerebral edema, cerebral herniation, seizure, new ischemic stroke.

6.6. Marketing Authorisation for Acute Ischemic Stroke

Alteplase has the marketing authorisation for the treatment of acute ischemic stroke within 3 hours of symptom onset by the US Food and Drug Administration (FDA) in 1996 and by the European Agency for the Evaluation of Medicinal Products (EMA) for all member states in September 2002. In 2011 15 European countries have approved intravenous thrombolysis with Alteplase for acute stroke up to 4.5 hours based on the mutual recognition procedure.

The approval of Alteplase as a marketed drug for acute ischemic stroke was based on the results of two placebo-controlled, double-blind trials (The NINDS t-PA Stroke Trial, Part 1 and Part 2) in patients with acute ischemic stroke. In both studies patients with neurological

deficit who could complete screening and begin study treatment within 3 hours from symptom onset were enrolled. As regards brain imaging, a cranial computerized tomography (CT) scan was performed prior to treatment to rule out intracranial haemorrhage. For details of the study protocol see the original publication (NINDS study group, 1995).

Table 1: The NINDS-tPA Stroke Trial, Part 2, 3-Month Efficacy Outcomes (NINDS study group, 1995)

	Frequency of Favorable Outcome[a]				
Favorable Outcmome defined by:	Placebo (n=165)	Alteplase (n=168)	Absolute Difference (95%CI)	Relative Frequency[b] (95%CI)	p-Value[c]
Generalized Estimating Equations (Multivariate)	-	-	-	1.34 (1.05-1.72)	0.02
Barthel Index	37.6%	50.0%	12.4% (3.0-21.9)	1.33 (1.04-1.71)	0.02
Modified Rankin Scale	26.1%	38.7%	12.6% (3.7-21.6)	1.48 (1.08-2.04)	0.02
Glasgow Outcome Scale	31.5%	44.0%	12.5% (3.3-21.8)	1.40 (1.05-1.85)	0.02
NIHSS	20.0%	31.0%	11.0% (2.6-19.3)	1.55 (1.06-2.26)	0.02

[a] Favorable Outcome is defined as recovery with minimal or no disability.

[b] Value > 1 indicates frequency of recovery in favor of Alteplase treatment.

[c] p-Value for Relative Frequency is from Generalized Estimating Equations with log link.

The initial study (NINDS-Part 1, n=291) evaluated neurological improvement at 24 hours after stroke onset. The primary endpoint, the proportion of patients with a 4 or more point improvement in the National Institutes of Health Stroke Scale (NIHSS) score or complete recovery (NIHSS score = 0), was not significantly different between treatment groups. A secondary analysis suggested improved 3-month outcome associated with Alteplase treatment using the following stroke assessment scales: Barthel Index, Modified Rankin Scale, Glasgow Outcome Scale, and the NIHSS. A second study (NINDS-Part 2, n=333) assessed clinical outcome at 3 months as the primary outcome. A favorable outcome was defined as minimal or no disability using the four stroke assessment scales: Barthel Index (score ≥ 95), Modified Rankin Scale (score ≤ 1), Glasgow Outcome Scale (score = 1), and NIHSS (score ≤ 1). In this study, depending upon the scale, the favorable outcome of minimal or no disability occurred in at least 11 per 100 more patients treated with Alteplase than those receiving placebo (see Table 1).

Safety data indicated a significant increase in ICH following Alteplase treatment, particularly symptomatic ICH within 36 hours. There were no increases in Alteplase-treated patients

compared to placebo-treated patients in the incidences of 90-day mortality or severe disability (see Table 2).

Table 2: The NINDS-tPA Stroke Trial, Safety Outcomes (NINDS study group, 1995)

	Part 1 and Part 2 Combined		
	Placebo (n=312)	Alteplase (n=312)	p-Value[b]
All Cause 90-day Mortality	64 (20.5%)	54 (17.3%)	0.36
Total ICH[b]	20 (6.4%)	48 (15.4%)	<0.01
Symptomatic ICH	4 (1.3%)	25 (8.0%)	<0.01
Asymptomatic ICH	16 (5.1%)	23 (7.4%)	0.32
Symptomatic ICH within 36 hours	2 (0.6%)	20 (6.4%)	<0.01
New Ischemic Stroke (within 3 months)	17 (5.4%)	18 (5.8%)	1.00

[a] Within trial follow-up period. Symptomatic ICH was defined as the occurrence of sudden clinical worsening followed by subsequent verification of ICH on CT scan. Asymptomatic ICH was defined as ICH detected on a routine repeat CT scan without preceding clinical worsening.

[b] Fisher's exact test

6.7. Post Marketing Authorisation Experience in Acute Ischemic Stroke

The Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST) has provided further evidence for the safety and efficacy of intravenous thrombolysis with Alteplase in acute ischemic stroke (Wahlgren et al., 2007). In this study with a sample of 6843 patients recruited from 285 centres in 14 European countries the proportion of patients with symptomatic intracranial haemorrhage (per the SITS-MOST protocol) was 1.7% (107/6444; 95% CI 1.4-2.0); at 7 days, the proportion with the same condition as per the Cochrane definition was 7.3% (468/6438; 6.7-7.9) compared with 8.6% (40/465; 6.3-11.6) in the pooled randomised controlled trials. The mortality rate at 3 months in SITS-MOST was 11.3% (701/6218; 10.5-12.1) compared with 17.3% (83/479; 14.1-21.1) in the pooled randomised controlled trials. Taken together, this study confirms in large data set that intravenous Alteplase is safe and effective in routine clinical use.

Recently, the third European Cooperative Acute Stroke Study (ECASS III) provided evidence for efficacy and safety of treatment with intravenous Alteplase administered between 3 and 4.5 hours after the onset of symptoms in a randomized controlled trial with a total of 821 patients (Hacke et al., 2008). In this trial, favourable outcome was more frequent in patients treated with Alteplase than with placebo (52.4% vs. 45.2%). In the global analysis, the outcome was also improved with Alteplase as compared with placebo (odds ratio, 1.28; 95% CI, 1.00 to 1.65; P<0.05). The incidence of intracranial haemorrhage was higher with Alteplase than with placebo (for symptomatic intracranial haemorrhage, 2.4% vs. 0.2%;

p=0.008). The study also did not find any significant difference in the rate of other serious adverse events.

7. Organisational Structure

7.1. Funding

WAKE-UP is an investigator initiated trial; sponsor is the University Medical Center Hamburg-Eppendorf (Universitätsklinikum Hamburg-Eppendorf, UKE). WAKE-UP will be funded by the European Commission (EC) as a collaborative project involving 13 European partners within FP7 (Project Number Health-F2-2011-278276). The manufacturer of Alteplase in Europe, Boehringer Ingelheim, has repeatedly denied any interest in funding a trial involving Alteplase in wake-up stroke. In order to avoid any potential conflict of interest or influence by the manufacturer of Alteplase, the study medication in WAKE-UP will be provided by an independent SME.

7.2. Overall Structure

The study will be conducted in approximately 60 centres in six European countries (BEL, DEN, ESP, FRA, GBR, GER). The clinical trial will be organised by a central coordinating centre located with the sponsor (UKE) and six national coordinating centres (see Figure). Only centres with a high volume of acute stroke patients, stroke MRI available as first line diagnostic tool, and experience with clinical trials in acute stroke will be involved as recruiting centres in order to ensure highest quality of acute stroke treatment and performance in the clinical trial. All recruiting centres will provide an infrastructure for the emergency assessment and treatment of acute ischemic stroke patients according to national and international guidelines. This comprises the availability of stroke MRI for acute stroke diagnostic with neuroradiological expertise and an experienced stroke neurologist 24/7, monitoring of patients on a specialised stroke unit, an optimised organisational structure allowing for the enrolment of patients into acute stroke clinical.

Orion Clinical Services Ltd, Berkshire, UK (ORION), an experienced Contract Research Organization (CRO), will be responsible for the monitoring of the study. Data management will be provided by Bioskin GmbH, Hamburg, Germany. Data will be recorded in an electronic Case Record Form (e-CRF) provided by Quadratek Data Solutions Ltd, Hampshire, UK. Study medication will be provided by ZytoService, Hamburg, Germany, a company experienced in the manufacture of medicinal products for clinical studies.

An independent Biostatistician will perform all statistical analysis.

All contact information will be provided in the investigator folder.

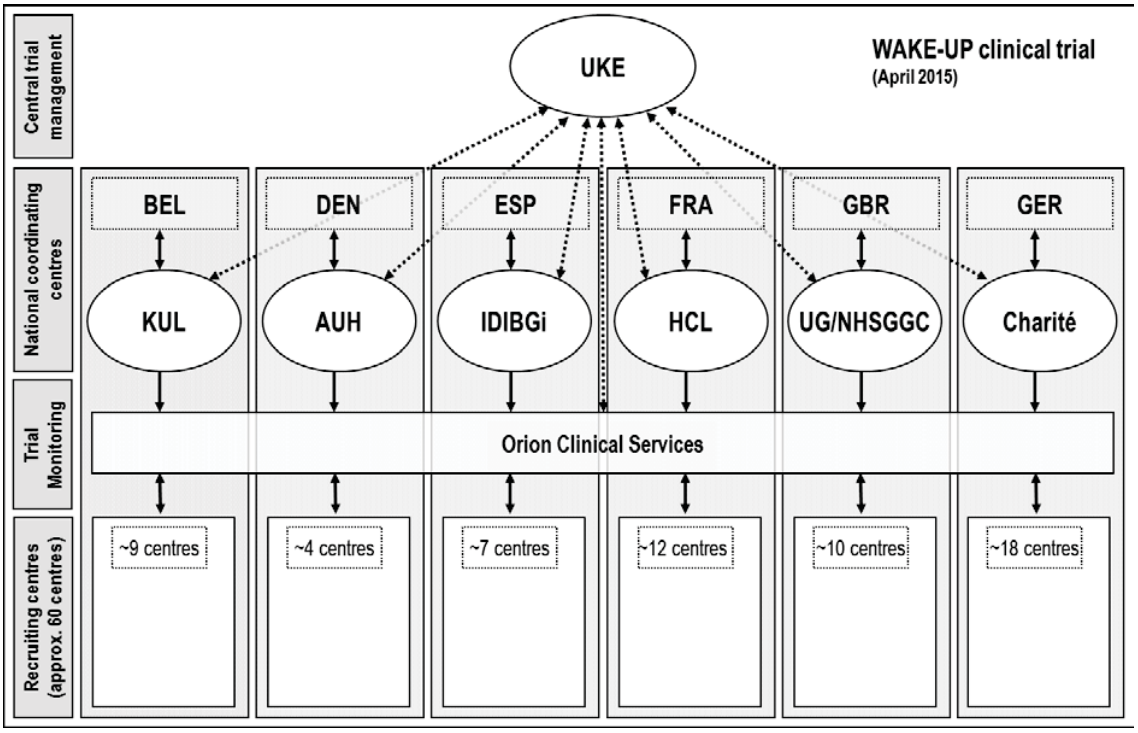


Figure: Organizational structure of the clinical trial

7.3. Trials Boards and Committees

The following boards and institutions will assure the success of the clinical trial:

7.3.1. Steering Committee

The Steering Committee (SC) of the clinical trial will decide on the final protocol and oversee the trial.

7.3.2. Data Safety and Monitoring Board

The independent Date and Safety Monitoring Board (DSMB) will regularly monitor the safety of the trial and guarantee the safety of patients at all stages of WAKE-UP. The members of the DSMB are not participants of the WAKE-UP consortium and not involved in the clinical trial in any other way. The DSMB may at any time recommend an amendment to the clinical trial protocol or termination of the trial in case of safety concerns. The tasks of the DSMB will be described in detail in a separate DSMB charter.

7.3.3. Ethics Advisory Board

The Ethics Advisory Board (EAB) will oversee the trial and guarantee that the trial is performed according to ICH-GCP guidelines and to international, European and national legislation. The EAB may at any time recommend an amendment to the clinical trial protocol or termination of the trial in case of ethical concerns.

7.3.4. Central Image Reading Board

The Central Image Reading Board (CIRB) will centrally review all images and provide reference judgements for the definition of the per protocol population and intracranial haemorrhages. The tasks of the CIRB will be described in detail in a separate CIRB charter.

7.3.5. Scientific Advisory Board

The Scientific Advisory Board (SAB) represented by world leaders in the field of stroke research and the conduction of acute stroke trials will provide a direct link between the WAKE-UP consortium and the wider scientific community.

8. Design

WAKE-UP is be an interventional, treatment, randomised, double-blind, placebo-controlled, parallel assignment, international, multi-centre efficacy and safety study. Patients will be randomized 1:1 to either treatment (Alteplase, rtPA) or placebo.

9. Population

Patients with acute ischemic stroke proven by MRI and unknown time from symptom onset which otherwise fulfil the approval criteria for intravenous thrombolysis in acute stroke represent the target population for WAKE-UP.

9.1. Inclusion Criteria:

Patients may be enrolled in the study if they meet all of the following clinical and imaging inclusion criteria:

9.1.1. Clinical Inclusion Criteria

- Clinical diagnosis of acute ischemic stroke with unknown symptom onset (e.g., stroke symptoms recognized on awakening)
- Last known well (without neurological symptoms) >4.5 hours of treatment initiation
- Measurable disabling neurological deficit (defined as an impairment of one or more of the following: language, motor function, cognition, gaze, vision, neglect)
- Age 18-80 years
- Treatment can be started within 4.5 hours of symptom recognition (e.g., awakening)
- Written informed consent from patient or proxy (see also Chapter Ethical Considerations, Informed Consent)

- Applicable for France only: subjects covered by or having the right to social security

9.1.2. Imaging Inclusion Criteria:

- Acute stroke MRI including diffusion weighted imaging (DWI), fluid attenuated inversion recovery (FLAIR), and MR-angiography (MRA) completed and showing a pattern of “DWI-FLAIR-mismatch”, i.e. acute ischemic lesion visibly on DWI (“positive DWI”) but no marked parenchymal hyperintensity visible on FLAIR (“negative FLAIR”) indicative of acute ischemic lesions ≤4.5 hours of age

9.2. Exclusion Criteria

Patients meeting any of the following clinical or imaging criteria will be excluded from study enrolment:

9.2.1. Clinical Exclusion Criteria

- Planned or anticipated treatment with endovascular reperfusion strategies (e.g. intra-arterial thrombolysis, mechanical recanalization techniques)
- Pre-stroke disability (inability to carry out all daily activities, requiring some help or supervision, i.e. slight disability corresponding to an MRS score >1)
- Participation in any investigational study in the previous 30 days
- Severe stroke by clinical assessment (e.g. NIHSS >25)
- Hypersensitivity to Alteplase or any of the excipients
- Pregnancy or lactating (formal testing needed in woman of childbearing potential; childbearing potential is assumed in women up to 55 years of age)
- Significant bleeding disorder at present or within past 6 months
- Known haemorrhagic diathesis
- Manifest or recent severe or dangerous bleeding
- Known history of or suspected intracranial haemorrhage
- Suspected subarachnoid haemorrhage (even if CT is normal) or condition after subarachnoid haemorrhage from aneurysm
- History of central nervous system (CNS) damage (e.g. neoplasm, aneurysm, intracranial or spinal surgery)
- Recent (within 10 days) traumatic external heart massage, obstetrical delivery, recent puncture of a non-compressible blood-vessel
- Current effective use of anticoagulants (e.g. Phenprocoumon, Warfarin, or new direct oral anticoagulants such as Apixaban, Dabigatran or Rivaroxaban) or current use of heparin and elevated thromboplastin time (low-dose subcutaneous

heparin is allowed); inclusion may be considered in patients using vitamin K-antagonists (Phenprocoumon or Warfarin) when appropriate tests of anticoagulant activity (INR) show no clinically relevant activity

- Platelet count <100.000/mm^3 (<100G/l)
- Blood glucose <50 or >400 mg/dl (<2.8 or 22.2 mmol/l)
- Severe uncontrolled hypertension, i.e. systolic blood pressure >185 mmHg or diastolic blood pressure >110 mmHg or requiring aggressive medication to maintain blood pressure within these limits (routine medical treatment is allowed to lower the blood pressure below these limits)
- Manifest or recent bacterial endocarditis, pericarditis
- Manifest or recent acute pancreatitis
- Documented ulcerative gastrointestinal disease during the last 3 months, oesophageal varices, arterial aneurysm, arterial/venous malformations
- Neoplasm with increased bleeding risk
- Manifest severe liver disease including hepatic failure, cirrhosis, portal hypertension and active hepatitis
- Major surgery or significant trauma in past 3 months
- Life expectancy 6 months or less by judgement of the investigator
- Any condition associated with a significantly increased risk of severe bleeding not mentioned above
- Any contraindication to MRI (e.g. cardiac pacemaker)

9.2.2. Imaging Exclusion Criteria:

- Poor MRI quality precluding interpretation according to the study protocol
- Any sign of intracranial haemorrhage on baseline MRI
- FLAIR showing a marked parenchymal hyperintensity in a region corresponding to the acute DWI lesion indicative of an acute ischemic lesion with a high likelihood of being > 4.5 hours old
- Large DWI lesion volume > 1/3 of the MCA or >50% of the anterior cerebral artery (ACA) or posterior cerebral artery (PCA) territory (visual inspection) or >100 ml
- Any MRI findings indicative of a high risk of SICH related to potential IV-tPA treatment in the judgment of the investigator

Inclusion and exclusion criteria mainly follow the license of Alteplase in Europe except for specific criteria related to the clinical trial (unknown time of symptom onset, MRI criteria, participation in other investigational studies). In addition, exclusion criteria used in WAKE-UP disregard three further exclusion criteria named in the license of Alteplase: minor stroke, prior stroke and diabetes, and seizure at onset of stroke. As a number of clinical studies has

reported poor outcome in patients that were excluded from thrombolysis due to “minor stroke” and a growing number of stroke experts has raised concerns against this exclusion criterion (Barber et al., 2001; Nedeltchev et al. 2007; De Keyser et al., 2007; Breuer et al., 2011) we decided not to exclude patients with minor stroke from enrolment in WAKE-UP. The results from a recent study based on SITS-MOST and VISTA data did not provide justification for the exclusion of patients with prior stroke and diabetes (Mishra et al., 2011). Thus, we decided to follow clinical practice in most stroke centers and not to exclude patients with prior stroke and diabetes from enrolment in WAKE-UP. Finally, the exclusion of patients with seizure at onset in previous CT-based trials and in the approval criteria resulted from concerns of treating stroke mimics with thrombolysis. However, in order to enroll a patient in WAKE-UP acute ischemic stroke has to be proven by MRI. Thus we can be sure that we will not enroll stroke mimics. Moreover, treatment with thrombolysis was found to be safe in patients with seizure at onset (Chernyshev et al. 2010; Tsivgoulis et al., 2011). In conclusion, we decided to follow clinical practice and not to exclude patients with seizure at onset from enrolment in WAKE-UP if they otherwise meet inclusion criteria and do not meet any other exclusion criteria.

Inclusion and exclusion criteria will be assessed based on information available during the screening period. There will be no retrospective exclusion of patients based on information coming to knowledge later on.

9.3. Sample Size

A total of 800 patients will be enrolled. Using DWI-FLAIR-mismatch as a criterion for enrolment, patients included are highly likely to be within a 4.5 h time window. As inclusion of patients within the first 1.5 hours of symptom onset will be rather unlikely, we expect a treatment effect comparable to that seen in patients treated 1.5-4.5 hours in the large stroke thrombolysis trials. In these trials a favourable outcome was observed in 43.3% of patients the treated with Alteplase as compared to 33.3% of patients in the placebo group (10.0% absolute difference) (Lees et al., 2010). Using these data for sample size calculation results in an estimated sample size of n=740 patients (n=370 per group) in order to demonstrate the expected treatment effect (i.e. to reject the null hypothesis of an identical proportion of favourable outcome in the two groups) with a type-1 error alpha = 0.05 (two-tailed) and 80% power (beta = 20%). Accounting for possible treatment failures, protocol violations and drop-outs, n=800 patients (n=400 per group) will be enrolled in WAKE-UP.

9.4. Sample Characteristics – Severity of Stroke Symptoms

We will aim to enrol a typical population of acute ischemic stroke patients with disabling neurological symptoms that are likely to benefit from treatment with intravenous Alteplase

given the published evidence. Clinical inclusion and exclusion criteria were defined in order to assure the selection of an adequate study population both requiring a measurable disabling neurological deficit and excluding severe strokes (e.g. defined by a score of the NIHSS exceeding 25). Together with the safety data we will monitor the baseline characteristics of the patients enrolled during the conduction of the trial. We will aim for a median NIHSS of 9-11 mirroring a population of acute stroke patients with on average moderate to severe symptoms comparable to the patients enrolled in the previous clinical trials of thrombolysis in acute stroke. In case of a median NIHSS outside this expected range the Steering Committee might consider a modification of the clinical inclusion criteria (e.g. specify a lower threshold on the NIHSS) to foster the enrolment of an adequate sample of patients.

10. Imaging

10.1. Rationale

For detailed imaging inclusion and exclusion criteria see above. The imaging inclusion criteria represent a crucial part of the trial. The presence of DWI-FLAIR-mismatch identifies patients with ischemic lesions less than 4.5 hours old. This, in turn, assures the enrolment of patients that are likely to benefit from thrombolysis. Imaging criteria rely on the DWI-FLAIR-mismatch pattern and incorporate findings from recent pilots studies by two WAKE-UP partners (Ebinger et al., 2010a; Thomalla et al., 2009) as well as from a large prospective observational study conducted with the Stroke Imaging Repository (STIR) Group under coordination of the WAKE-UP coordinators (PRE-FLAIR) (Thomalla et al., 2011). See Appendix A for imaging examples. Imaging exclusion criteria mainly aim at the reduction of the risk of SICH and involve the exclusion of large DWI lesion which were found to be associated with increased risk of SICH in previous studies conducted with participation of WAKE-UP consortium partners (Singer et al., 2008).

Further details on the imaging inclusion and exclusion criteria will be specified in the imaging handbook. This handbook will include a set of example images illustrating imaging inclusion and exclusion criteria of the trial.

10.2. MRI Protocol

The following MRI sequences are mandatory:

- DWI
- FLAIR
- Sequences sensitive to haemorrhage, e.g. T2*-weighted gradient recalled echo (GRE) sequences

- Time-of-flight (TOF) MR-angiography

DWI, FLAIR, an MRI sequence to exclude haemorrhage, and a time of flight (TOF) MR-angiography (MRA) are mandatory. Additional sequences including PI are not required but any additional imaging data will be collected if available. Information on vessel occlusion or perfusion is not used for patient enrolment, but information on the vessel status will be used for secondary endpoint analysis and also may be used by the DSMB to weigh results of safety analyses. Only patients with a completed MRI according to study protocol are eligible. Contraindications against MRI as well as bad quality of MRI exclude patients from the trial.

Details on the MRI protocol including sequence parameters and quality requirements will be specified in an imaging handbook provided together with the investigator brochure. The CIRB will continuously monitor the fulfilment of the pre-specified MRI standards in each participating centre.

10.3. Training

Investigators involved in image reading within the trial will participate in a standardized image reading training before study start.

11. Randomization and Unblinding

Each patient is uniquely identified in the trial by a combination of his centre number and patient number. The centre number is assigned to the recruiting centre. The patient number is assigned to the patient by the investigator. Patient numbers start with number 01 in each centre and subsequent patients are assigned consecutive numbers. Randomization will be done by a computerized central interactive web response system (IWRS). The automated system will assign an appropriate set of study medication to each patient.

Eligible patients are randomized to treatment with either Alteplase or placebo with a ratio of 1:1. Patients are randomized in four cohorts stratified according to age (≤60/>60 years) and severity of symptoms (NIHSS ≤10/>10). The approach of stratified randomisation helps to avoid potential imbalances between the groups that might affect outcome and bias results (Stanley, 2007) and has already been used in stroke trials, e.g. the glycine antagonist in neuroprotection (GAIN) trials (Lees et al., 2000). Age and severity of symptoms as measured by the NIHSS are known to represent two major covariate predictors of outcome in acute stroke (Weimar et al., 2004). Thus, we stratify randomisation by age and pre-treatment NIHSS score.

Both, patient and investigator are blinded to the type of treatment. The investigator will be provided with the options to selectively unblind an individual patient 24/7 if deemed necessary for any clinical reasons. Premature breaking of the treatment code should be

restricted to cases of emergency where knowledge of thy type of treatment is considered necessary for adequate treatment of the patient. The sponsor must be notified about any patient unblinded immediately.

12. Treatment

Study medication will be provided by ZytoService Deutschland GmbH, Hamburg, Germany. Zytoservice are experts in the manufacture of medicinal products for clinical studies and specialised in the manufacture of sterile products like injections and infusions as unique preparations for individuals:

ZytoService Deutschland
GmbH Albert-Schweitzer
Ring 18
22045 Hamburg Germany

All study medication will be manufactured, tested, released, and shipped according to Good Manufacturing Practice (GMP) guidelines.

The supplied study medication will be provided in 50 ml vials of identical appearance containing lyophilisate together with 50 ml vials of sterile water for injections. Labelling and packaging of study medication will be conducted according to GMP, GCP, and any local or national regulatory requirements.

Treatment has to be initiated as soon as possible within 60 minutes of the end of the MRI examination.

12.1. Study drug

Patients randomized to the active study drug will receive intravenous Alteplase 0.9 mg/kg body-weight up to a maximum of 90 mg, 10% as bolus, 90% over 1 hour as infusion. The drug will be supplied as provided by the manufacturer in unopened but relabelled vials as a lyophilised powder to be reconstituted as a solution. Accordingly, patients randomized to the placebo arm will receive intravenous placebo 0.9 mg/kg body-weight up to a maximum of 90 mg, 10% as bolus, 90% over 1 hour as infusion.

12.2. Placebo

The matching placebo is in a form of a lyophilised powder to be reconstituted to obtain a solution indistinguishable from the active drug.

12.3. Drug Accountability

Study Sites will be provided with sufficient amounts of study medication. The study medication must not be used outside the study protocol. The investigator or authorized staff is obliged to document the receipt, dispensation, and return of all study medication received during the study. The Investigator has the overall responsibility for administering/dispensing the study medication. Where permissible, tasks may be delegated to a qualified designee who is adequately trained on the protocol. The delegation must be documented on the applicable delegation form.

The Sponsor or its representative must be permitted access to review the supplies storage and distribution procedure and records.

12.4. Concomitant Treatment

All patients will be treated according to European and national guidelines and recommendations of acute stroke treatment (ESO, 2008). Treatment with intravenous thrombolysis or endovascular recanalization strategies excludes the patient from participation in the trial (see Exclusion Criteria).

Administration of heparin, low molecular weight heparin, thrombocyte aggregation inhibitors (platelet inhibitors, e.g. Aspirin, Clopidogrel, Ticlopidine) and anticoagulants (e.g. Phenprocoumon, Warfarin, Dabigatran, Apixaban, Rivaroxaban) is prohibited for 24 hours after treatment with the study drug.

Any concomitant medication within one week prior to enrolment and all concomitant medication during the course of the study must be documented in the eCRF.

13. Study Schedule

For an overview of the study schedule see Chapter 2. Flow Chart and Schedule of Assessments. The study consists of three periods: Screening (V0), Treatment (V1, V2, V3), and Follow Up (V4, V5).

13.1. Screening Period (Visit V0, Randomization)

13.1.1. Visit V0

For each patients deemed eligible for the study by the investigator written informed consent will be obtained from the patient or the patient’s representative as agreed upon with the Ethics Committee according to national requirements prior to the performance of any protocol-related investigation.

During the screening period the following assessments will be performed in patients for whom written informed consent has been obtained:

- Collection of demographic data and medical history
- Physical examination
- Determination of the pre-stroke MRS by interview of the patient or his kin
- Assessment of the neurological deficit using the NIHSS (see chapter Assessments)
- Collection of information on previous medication
- Measurement of blood pressure and heart rate
- Measurement of body temperature
- Screening laboratory tests by the local laboratory including the International Normalized Ration (INR), haematology with Haemoglobin, White Blood Cell count and Platelet count, Serum glucose
- Pregnancy test in women of childbearing potential (urinary test)
- A brain MRI scan according to the WAKE-UP MRI protocol (see chapter Imaging)
- A 12-lead ECG
- Documentation of any previous and concomitant medication
- Assessment of Adverse Events
- Checking of inclusion and exclusion criteria

13.1.2. Randomization (R)

If all inclusion criteria are met and no exclusion criteria are present randomization will be performed (see chapter Randomization).

13.2. Treatment Period (Visits V1, V2, V3)

After randomization is completed the treatment period starts which comprises the treatment with the study drug (V1), 24h monitoring (V2) and the clinical and MRI examination 22-36 h after treatment (V3).

13.2.1. Visit V1

Before administration of the study drug the following assessments have to be performed:

- Measurement of blood pressure and heart rate

The study medication will be administered as specified for intravenous Alteplase (see Summary of Product Characteristics [Fachinformation]). The study medication has to be administered as soon as possible and not later than 60 minutes after completion of the MRI study.

During the administration of the study drug blood pressure and heart rate will be measured every 15 minutes. Once again, any concomitant medication will be documented and Adverse Events will be assessed.

13.2.2. Visit V2

Visit V2 comprises the period of 24h monitoring of the patient between the administration of the study drug and Visit 3. During this period the following assessments will be made:

- Measurement of blood pressure and heart rate every hour
- Measurement of body temperature every 6 hours
- Documentation of any concomitant medication
- Assessment of Adverse Events

In any case of neurological deterioration judged as significant by the investigator an assessment of the neurological deficit using the NIHSS and another brain scan has to be performed in order to look for intracranial haemorrhage.

The 24h monitoring should be performed on a specialized unit for the treatment of acute stroke patients (e.g. Stroke Unit, Neurological Intensive Care Unit).

13.2.3. Visit V3

Visit 3 comprises the clinical examination and a second MRI scan which is performed to delineate the infarct volume and to diagnose intracranial haemorrhage. Visit 3 will be done 22-36 h after treatment. The following assessments will be made:

- Physical examination
- Assessment of the neurological deficit using the NIHSS (see chapter Assessments)
- Measurement of blood pressure and heart rate
- Measurement of body temperature
- Screening laboratory tests by the local laboratory including the International Normalized Ration (INR), haematology with Haemoglobin, White Blood Cell count and Platelet count, Serum glucose
- A brain MRI scan according to the WAKE-UP MRI protocol (see chapter Imaging)

- Documentation of any concomitant medication
- Assessment of Adverse Events

13.3. Follow Up Period (Visits V4, V5)

The Follow Up period covers the period from the subacute stage (day 5-9 or hospital discharge) in Visit 4 to the final follow up examination 80-100 days after stroke which defines the primary endpoint in Visit 5.

13.3.1. Visit V4

The following assessments will be performed:

- Physical examination
- Assessment of the neurological deficit using the NIHSS (see chapter Assessments)
- Measurement of blood pressure and heart rate
- Measurement of body temperature
- Documentation of any concomitant medication
- Assessment of Adverse Events

13.3.2. Visit V5

The following assessments will be performed:

- Physical examination
- Assessment of the neurological deficit using the NIHSS (see chapter Assessments)
- Assessment of functional status using the MRS and BI (see chapter Assessments)
- Assessment of outcome using the GOS (see chapter Assessments)
- Assessment of depressive symptoms using the BDI (see chapter Assessments)
- Assessment of functional health status and quality of life using the EQ-5D (see chapter Assessments)
- Use of health care system resources using a questionnaire (see chapter Assessments)
- Documentation of any concomitant medication
- Assessment of Adverse Events

14. Assessments

Neurological symptoms will be assessed using the National Institutes of Health's Stroke Scale (NIHSS) (See Appendix B). Functional outcome will be assessed using the modified Rankin Scale (MRS) (see Appendix C), the Barthel Index (BI) (see Appendix D), and the Glasgow Outcome Scale (see Appendix E). All neurological assessments will be performed by certified examiners.

General physical examination will be performed covering all relevant systems. This will include an estimation of the body weight which is necessary for the calculation of the dose of the study drug to be administered.

A standard 12-lead ECG will be performed.

For laboratory tests the results are taken from the local routine laboratory test.

Infarct volume will be measured on follow-up MRI after 22-36 hours. The infarct lesion will be measured using an interactive semi-automatic thresholding procedure applied on DWI as reported in a preparatory study (Thomalla et al., 2011).

Depressive symptoms will be assessed using the Beck Depression Inventory (BDI) (see Appendix F). Functional health status and Quality of life will be assessed using the EQ-5D (see Appendix G). Use of health care system resources will be recorded using a questionnaire (see Appendix H).

15. Endpoints of the Clinical Trial

15.1. Efficacy Analysis – Primary Endpoint

Primary endpoint is "favourable outcome" defined by a score of 0-1 on the modified Rankin Scale (MRS) 90 (±10) days after stroke.

We will use „favourable outcome" as primary efficacy endpoint, as it defines the most relevant clinical outcome for previously not disabled acute stroke patients. A score of 0-1 on the MRS mirrors no or only a minimal neurological deficit, thus in a way a "cure" of the acute stroke symptoms. Moreover, favourable outcome was the primary efficacy outpoint measure in any acute large stroke thrombolysis trial: NINDS (NINDS study group, 1995), ECASS II (Hacke et al., 1998), ECASS III (Hacke et al., 2008), EPITHET (Davis et al., 2008). Favourable outcome is also the primary endpoint in the currently conducted trial of MRI guided thrombolysis based on penumbral imaging patterns (EXTEND). Thus, the use of this endpoint will make the results of WAKE-UP comparable to those of previous and current stroke thrombolysis trials.

15.2. Efficacy Analysis – Secondary Endpoints

The following secondary endpoints will be studied:

- Global Outcome Score (combination of MRS 0-1, NIHSS 0-1, Barthel Index (BI) 95-100, Glasgow Outcome Scale (GOS)1) 90 (±10) days after stroke
- Categorical shift in MRS 90 (±10) days after stroke
- Responder analysis relating MRS 90 (±10) days after stroke to baseline NIHSS score: “response” defined by NIHSS <7 = MRS 0; NIHSS 8-14 = MRS 0-1; NIHSS >14 = MRS 0-2
- Infarct volume after 22-36 hours
- Depressive symptoms 90 (±10) days after stroke (BDI)
- Functional health status and quality of life 90 (±10) days after stroke (EQ-5D)
- Use of health care system resources (questionnaire)

A number of different secondary efficacy endpoints have been used in previous stroke thrombolysis trials. A global outcome score defined by the favourable outcome scores on the NIHSS (score 0-1), BI (score 95-100), and GOS (score 1) 90 days after stroke was previously in NINDS trial and ECASS trials (NINDS study group, 1995; Hacke et al., 2008; Hacke et al., 1998). The use of a categorical shift in MRS 90 days after stroke has recently been suggested to provide a more comprehensive index of the clinical impact of an acute stroke treatment (Saver, 2007). This accounts for the fact that stroke treatment usually is not curative, but rather has the potential to improve patient outcome over the whole range of functional measurements. A different approach to account for the impact of initial stroke severity on functional outcome is the definition of treatment response in relation to the initial symptom severity (responder analysis), as it was suggest in AbESTT-II (Adams et al., 2008a).

Final ischemic lesion volume has been used as a surrogate marker of stroke outcome in previous trials and contributed to an improved understanding of the effects of acute stroke treatment as well as to a refinement of selection criteria to identify patients for thrombolysis. We will measure infarct volume on follow-up MRI after 22-36 hours. This may be a rather early time point to measure final infarct volume as relevant lesion growth might occur beyond 22-36 hours of treatment. However, excellent correlation of early lesion volume measurements with final infarct volume measured 90 days after stroke (Ebinger et al. 2009). Infarct lesion volume will be measured centrally by investigators blinded to the clinical data of patients.

Depressive symptoms are frequent after ischemic stroke and can have a major impact on functional impact and quality of life. We will use the BDI to study depressive symptoms at the final outcome examination 90 (±10) days after stroke. Both absolute values and the proportion of patients reaching a score within the predefined categories of minimal depression, mild depression, moderate depression, and severe depression will be looked at.

Little is known about the impact of treatment with intravenous thrombolysis on quality of life after stroke. As a secondary endpoint we will also assess functional health status and quality of life 90 (±10) days after stroke using the EQ-5D. Both the summary index (EQ-5D Index) and the proportion of patients with good quality of life according to a pre-specified cut-off (EQ-5D Index ≥70) will be studied.

The use of health care system resources will be asked for using a simple questionnaire.

15.3. Safety Analysis – Primary Endpoints

The following primary safety endpoints will be studied:

- Mortality 90 (±10) days after stroke
- Death or dependency 90 (±10) days after stroke (MRS 4-6)

Mortality and the combined endpoint of death or dependency (defined as an MRS score of 4-6) after 90 (±10) days will be the primary safety endpoints of the WAKE-UP clinical trial. In the pooled analysis of 3669 patients from the randomized trials of thrombolysis in stroke mortality was 11.9% in patients treated with placebo and 13.9% in patients treated with Alteplase within 6 hours of symptom onset (p=0.1080) (Lees et al., 2010). For patients treated within 4.5 hours mortality was 12.7% for the placebo group and 13.4% for the Alteplase group.

15.4. Safety Analysis – Secondary Endpoints

In addition to the clinical endpoints, we will look at intracranial haemorrhage being the most feared complication of thrombolysis in acute stroke:

- Symptomatic intracranial haemorrhage (SICH) as defined in SITS-MOST
- SICH as defined ECASS II
- SICH as defined in NINDS
- Parenchymal haemorrhage type 2 (PH-2)

There has been some debate within the community of stroke researches during the last years as towards the optimal definition of SICH. We will use the definition of SICH as defined in SITS-MOST: “local or remote parenchymal hematoma type 2 on the imaging scan

obtained 22 to 36 hours after treatment, plus neurologic deterioration, as indicated by a score on the NIHSS ≥ 4 points than the baseline value or the lowest value between baseline and 24 hours, or haemorrhage leading to death" (Wahlgren et al., 2007). This definition assures to include only relevant large intracranial haemorrhages likely to be responsible for clinical deterioration, i.e. "truly" symptomatic ICH. The rate of SICH as defined in SITS-MOST was 1.9% in ECASS III (Hacke et al., 2008), 1.7% in SITS-MOST (1.7%) (Wahlgren et al., 2007), 1.7% for 21204 patients treated within 3 hours, and 2.2% for 2317 patients treated 3-4.5 hours of stroke onset registered in SITS-ISTR (Ahmed et al., 2010). We will also look at different definitions of SICH including those used in ECASS II ("any hemorrhage with neurologic deterioration, as indicated by an NIHSS score ≥ 4 points than the value at baseline or the lowest value in the first 7 days, or any hemorrhage leading to death") (Hacke et al., 1998), which is the primary endpoint in MR WITNESS, and the definition used in the NINDS stroke thrombolysis trial ("any new hemorrhage associated with any neurological deterioration") (NINDS study group, 1995).

Intracerebral haemorrhage will also be classified according to the radiologic criteria suggested in ECASS (Hacke et al., 1995): haemorrhagic infarction type 1 (HI-1), haemorrhagic infarction type 2 (HI-2), parenchymal haemorrhage type 1 (PH-1), and parenchymal haemorrhage type 2 (PH-2). The frequency of PH-2 (defined as blood clots exceeding 30% of the infarct area with substantial space occupying effect) will be studied and compared to previous trials of stroke thrombolysis. The rate of PH-2 was 5.2% in the updated pooled thrombolysis trials analysis (Lees et al., 2010).

15.5. Safety Analysis – Adverse Events

15.5.1. Adverse Events – Definition and Causality Assessment

Any adverse change in health or the appearance of or worsening of any undesirable sign, symptom or medical condition occurring after enrolment into the trial will be documented as Adverse Event (AE) whether or not it is considered to be related to the study drug by the Sponsor or Investigator. An adverse event also includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

In this study, the collection of adverse events starts immediately after the subject signs the informed consent document. The occurrence of Adverse Events will be explored at each visit by questioning and examination of the patient. Adverse Events occurring between two visits will also be asked for and recorded.

15.5.1.1. Severity Categorization

The medical assessment of severity of AEs is determined by using the following definitions:

Mild (grade 1): A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate (grade 2): A type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.

Severe (grade 3): A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Life-threatening (grade 4): Substantial risk of dying at time of event

Death (grade 5)

15.5.1.2. Relationship Categorization

An Investigator must make the assessment of relationship to investigational product for each AE. The Investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the investigational product. If there is no valid reason for suggesting a relationship, then the AE should be classified as ‘not related’. Otherwise, if there is any valid reason, even if undetermined or untested, for suspecting a possible cause and effect relationship between the investigational product and the occurrence of the AE, then the AE should be considered ‘related’. The causality must be documented in the source document.

The following additional guidance may be helpful:

Term	Relationship	Definition
Related	Yes	The temporal relationship between the event and the administration of the IMP is compelling and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the subject's medical condition, other therapies, or accident.
Not Related	No	The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident and no plausible temporal or biologic relationship exists between the investigational product and the event.

15.5.2. Serious Adverse Events

A Serious Adverse Event (SAE) is any untoward medical occurrence (whether considered to be related to investigational product or not) that at any dose:

- Is fatal or life-threatening
- Requires or prolongs hospitalization
- Results in persistent or significant disability/incapacity
- Constitutes a congenital anomaly or birth defect
- Requires intervention to prevent permanent impairment or damage
- Is an Important Medical Event;

Note: Important Medical Events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization; or the development of drug dependency or drug abuse.
Hospitalizations which are the result of elective or previously scheduled surgery for pre-existing conditions which have not worsened after initiation of treatment should not be classed as SAEs. For example, an admission for a previously scheduled ventral hernia repair would not be classed as an SAE. However, complication(s) resulting from a hospitalization for an elective or previously scheduled surgery that meets serious criteria must be reported as an SAE(s).

15.5.3. Adverse Event Recording and Follow Up

The investigator is responsible for the evaluation and report of any AE occurring during the study. The investigator will record onset, duration, intensity, any taken action,

evolution/outcome, and the causality assessment for any AE. Any AE resulting in withdrawal from the study and any AE persisting at the end of the study have to be followed up.

15.5.4. Serious Adverse Events Reporting

While AEs are recorded in the AE form of the e-CRF special reporting requirements apply for the reporting of SAEs. SAEs require immediate reporting.

The investigator shall notify within 24 hours the sponsor of any SAE that occurs in a subject at a trial site immediately at:

WAKE-UP SAFETY DESK

FAX: +33 4 72 11 51 90

ALL FORMS MUST BE DATED AND SIGNED BY AN AUTHORIZED INVESTIGATOR

The Investigator must complete, sign and date the Serious Adverse Event Form and verify the accuracy of the information recorded on the form with the corresponding source documents. SAE reports will be collected centrally at the safety desk which is located at HCL. A copy of all SAE reports will be send to the central trial management at CTC North.

Such preliminary reports will be followed by detailed descriptions later which will include anonymous copies of hospital case reports or pertinent results and other documents when requested and applicable.

All serious adverse events that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- the event resolves
- the event stabilizes
- the event returns to baseline, if a baseline value is available
- the event can be attributed to agents other than the study drug or to factors unrelated to study conduct
- when it becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts).

Any additional information known after the event has been initially reported should be sent using a new SAE report form. New information will be noted on the "serious adverse event" form, by ticking the box marked "follow-up" and sending to WAKE-UP SAFETY DESK.

Patients withdrawn from the study treatment due to any adverse event will be followed at least until the outcome is determined even if it implies that the follow-up continues after the patient has left the trial.

The investigator shall assess the seriousness of the adverse event. This is based on the regulatory definitions of seriousness as defined in Section 15.4.2. The investigator shall assess the causality of the serious adverse event (see Section 15.4.1.2). This is a clinical assessment of whether the adverse event is likely to be related to the trial drug. The sponsor is responsible for assessing seriousness, causality and expectedness of the serious adverse event. The evaluation of the expectedness is based on knowledge of the adverse reaction and the reference document.

Reference document for medicinal product: Summary of Product Characteristics (SmPC) of Actilyse® (Alteplase).

Reference document for placebo: not applicable.

An unexpected serious adverse event is an adverse event of which the nature or severity is not consistent with the reference document (Suspected Unexpected Adverse Reaction, SUSAR).

The sponsor will be responsible for expedited reporting (SUSAR, New Safety Issues, Annual safety Reports) to the relevant Competent Authorities and to the Ethics Committees in accordance with the EU Clinical Trials Directive (2001/20/CE) and local regulation.

16. Study Discontinuation Criteria

16.1. Discontinuation of individual patients

The treatment with the study drug should be terminated immediately under the following circumstances:

- In case of any serious bleeding (not controllable by local pressure).
- In case of uncontrollable arterial hypertension occurring after the initiation of treatment (arterial blood pressure > 185 mmHg).
- In case of anaphylactic reaction.
- If, in the investigators opinion, continuation of the study drug would be detrimental to the patient’s well-being.
- Withdrawal for personal reasons.

Discontinuation of treatment will not lead to discontinuation of the patient from the trial. Every effort will be made to complete follow-up examinations in all patients no matter if they have treated according to the trial protocol or not.

16.2. Discontinuation of the trial

The sponsor has the right to halt or terminate the study in any case of concern for the safety of the patients resulting from new information. Clinical trial data will be analysed sequentially for efficacy after enrolment of 500 and 800 patients and for safety after inclusion of 100, 200, 300, 500, and 800 patients.

Mortality and the combined criterion of death or dependency will be formally monitored for safety and the following predefined stopping rules will apply:

- Mortality: the stopping rule proposed to the DSMB is to stop the trial after an interim analysis if the proportion of death in the Alteplase group exceeds the one in the placebo group with a Chi-square test value greater than the threshold defined by the alpha-spending function (one sided test, overall alpha level of 0.10).
- Death or dependency: the stopping rule proposed to the DSMB is to stop the trial after an interim analysis if the proportion of the combined endpoint death or dependency (defined by an MRS score of 4-6) in the Alteplase group exceeds the one in the placebo group with a Chi-square test value greater than the threshold defined by the alpha-spending function (one sided test, overall alpha level of 0.10).
- SAE: the DSMB will continuously monitor the rate of SAE and SUSAR and is suggested to stop the trial in any case of unexpected high or alarming rates of SAE or SUSAR.

Interim analysis of the primary efficacy endpoint will be performed to test for overwhelming efficacy after enrolment of 500 patients.

- Overwhelming efficacy: the stopping rule proposed to the DSMB is to stop the trial after the interim analysis if the proportion of the primary endpoint (defined by a MRS score of 0-1) in the Alteplase group is significantly different from the one in the placebo group with a Chi-square test value greater than the threshold defined by the alpha-spending function (two sided test, overall alpha level of 0.05).

These stopping rules will be considered as guidelines and will not be binding to the DSMB. The decision to stop or continue the trial will be based on the overall assessment of risk and benefit.

The sponsor may terminate the study at a recruiting site when the investigator fails to comply with relevant regulations or insufficiently adheres to the clinical trial protocol.

17. Statistical Analysis

All analyses will be conducted on data from all randomly assigned patients, whether or not treated, according to the intention-to-treat principle. Analyses will also be repeated according to the per protocol principle. The judgements of central image reading will be used to define the population for per protocol analysis. One interim analysis of primary endpoint is planned after the inclusion of 500 patients (250 in each group), with a statistical stopping guideline for an overwhelming benefit.

Handling of missing data

All efforts will be made to collect outcome data also in patients withdrawn from the trial for whichever reasons and to minimize the amount of missing data. Sensitivity analyses based on different hypotheses about the missingness pattern of the primary outcome will be performed to test for the robustness of the primary analysis.

Analysis of Efficacy – primary endpoint

The primary efficacy endpoint is disability evaluated 90 (±10) days after stroke using the modified Rankin Scale (MRS), dichotomized in favourable (MRS 0-1) and unfavourable (MRS 2-6) outcome. One interim analysis of primary endpoint is planned after the inclusion of 500 patients (250 in each group), with a statistical stopping guideline for an overwhelming benefit. A Lan-DeMets alpha spending function will be used to control for the overall alpha level, using O'Brian and Fleming boundaries (corresponding to alpha-level of 0.0132 and 0.0460 at the interim and final analysis, respectively). Those values will be adapted depending on the effective number of patients analysed at the time of the analyses). Between-group differences will be tested using a chi-square test. An unconditional logistic regression model (Breslow and Day, 1980) will be fitted to estimate the odds-ratio associated with treatment effect, restricting the adjustment for the randomisation stratified factors (age and symptoms severity). Corresponding confidence intervals will be provided. In a complementary analysis, a more complete model will be fitted, retaining also (both clinical and imaging) baseline variables with p-value <0.10.

Analysis of Efficacy – secondary endpoints

Global Outcome Score analysis: The global outcome score analysis is a multidimensional calculation of a favourable outcome(Saver, 2007; Tilley et al., 1996), calculated from four dichotomised individual outcome scales recorded 90 (±10) days after stroke: 1) the Modified Rankin Scale (MRS 0-1);2) the National Institutes of Health Stroke Scale (NIHSS, score 0-1); 3) the Barthel Index (BI, score 95-100); 4) the Glasgow Outcome Scale (GOS, score 1). The global statistic allows taking into account the contribution to the health status of each outcome scale measurement. This statistical approach leads to perform a global odds-ratio

test based on a linear logistic-regression model – a method that uses generalised estimation equations to perform a Wald-type test – to compare the proportion of favourable outcome in the Alteplase arm and the placebo arm.

Responder analysis: Responder analysis adjusts outcome thresholds according to stroke severity at study entry (Adams et al., 2004; Saver, 2007). Patients with mild deficits at study entry (NIHSS <7) must attain MRS 0, patients with moderate deficits (NIHSS 8-14) must attain MRS 0-1, and patients with severe deficits (NIHSS >14) must attain MRS 0-2, respectively, to be considered as responders. Treatment effect will be analysed using the odds-ratio estimate and its 95% confidence interval.

Analysis of the categorical shift in MRS 90 (±10) days after stroke: The categorical shift in MRS 90 (±10) days after stroke(Saver, 2004) will be analysed fitting a log-linear model for ordinal data.

Infarct volume after 22-36 hours: infarct volume at follow-up after 22-36 hours will be measured by MRI and compared between the Alteplase arm and the placebo arm using a Student's t-test, the variable being transformed if necessary. A multivariate analysis will be performed fitting a linear regression model.

Depressive symptoms 90 (±10) days after symptom onset (BDI): BDI values will be compared between the Alteplase arm and the placebo arm both for absolute values and for the proportion of patients reaching scoring within the predefined categories of minimal depression, mild depression, moderate depression, and severe depression. Absolute values will be compared between the Alteplase arm and the placebo arm using a Student's t-test, the variable being transformed if necessary. A multivariate analysis will be performed fitting a linear regression model. The distribution of the categorized scores between the two arms will be compared fitting polytomous ordinal logistic regression models.

Functional health status and quality of life 90 (±10) days after stroke (EQ-5D): The summary index (EQ-5D Index) and the proportion of patients with good quality of life according to a pre-specified cut-off (EQ-5D Index ≥70) will be compared between the Alteplase arm and the placebo arm using a Student's t-test or a chi-square test as appropriate. An unconditional logistic regression model (Breslow and Day, 1980) will be fitted to estimate the odds-ratio associated with treatment effect.

Use of health care system resources: the use of health care system resources will be described for both groups and comparison performed using linear or generalized linear models, according to the type of variables.

Analysis of Safety:

Mortality and death or dependency 90 (±10) days after stroke will be analysed sequentially after inclusion of 100, 200, 300, 500, and 800 patients. A Lan-DeMets alpha spending function will be used to control for the overall alpha level, using Hwang-Shih-DeCani boundaries with parameter 1.2.The stopping rule proposed to the DSMB is to stop the trial after an interim analysis if the proportion of death, or the proportion of the combined endpoint death or dependency (defined by an MRS score of 4-6) in the Alteplase group exceeds the one in the placebo group with a Chi-square test value greater than the threshold defined by the alpha-spending function (one sided test, overall alpha level of 0.10 for each endpoint). The occurrence of SICH (as defined in SITS-MOST, ECASS II, NINDS) and PH-2 will also be compared between the test and the control arm but will not be used as endpoints for formal stopping rules. Formally, these variables will not be considered as sequentially analyzed. All safety variables being binary, an unconditional logistic regression model (Breslow and Day, 1980) will be fitted to estimate the odds-ratio associated with treatment effect,

Analysis of Baseline Symptom Severity:

In order to ensure the enrolment of a typical population of acute ischemic stroke patients with on average moderate to severe disabling neurological symptoms the severity of symptoms at baseline measured by the NIHSS will be analysed sequentially after inclusion of 100, 200, 500, and 800. Descriptive statistics (median, IQR, mean, SD) will be computed. In case of baseline symptom severity outside the expected range (e.g. a median NIHSS below or above the expected range of 9-11) the Steering Committee might consider a modification of the clinical inclusion criteria (e.g. specify a lower threshold on the NIHSS).

18. Study periods

The recruitment period of WAKE-UP is expected to be 45 months followed by a follow up period of 3 months. Patient enrolment is planned to start in June 2012.

Estimated start (first patient in):	June 2012
Estimated end of enrolment (last patient in):	February 2016
Estimated end of follow up (last patient out):	May 2016
Estimated end of trial (data base lock):	August 2016

19. Ethical and Regulatory Considerations

19.1. General Requirements and Considerations

Approval for the conduct of the trial will be obtained from the Ethics Committees of all participating centres as well as from the Regulatory Authorities in the six participating European countries (Belgium, Denmark, France, Germany, Spain, United Kingdom). The trial will not start in any country before written approval and authorisation by the respective Ethics Committee and Regulatory Authority. Any subsequent protocol amendment will be submitted to the Ethics Committees for approval.

The trial will be conducted according to the principles laid down

- Declaration of Helsinki in its version of Seoul, 2008;
- The EU Clinical Trial Directive 2001/20/EC;
- The "Note for Guidance on Good Clinical Practice" (CPMP/ICH/135/95 of January 17, 1997);
- The applicable national drug laws, e.g. German Drug Law (Arzneimittelgesetz, 15. Novelle, AMG);
- GCP-Regulation from August 9, 2004.

The involvement of the European patient's organisation Stroke Alliance For Europe (SAFE) in the clinical trial of WAKE-UP will further ensure that the patients safety has the highest priority at any stage of the trial.

Besides the treatment to be tested all diagnostic procedures and treatments applied are part of standard management of acute stroke patients and will follow European and national guidelines. These procedures are therefore of immediate benefit to the patients. The investigators will assure that each patient enrolled in the trial will receive best practice medical treatment.

19.2. Quality Control and Monitoring

The design of the trial has been carefully reviewed and approved by the Steering Committee of WAKE-UP before being submitted to for approval by the Ethics Committees and Regulatory Authorities. In addition, the independent EAB and DSMB and an external SAB have reviewed and approved the trial protocol and will continuously monitor the conduction of the trial. The composition of these boards assembling renowned independent experts will guarantee an adequate high alertness to all ethical and safety issues.

19.3. Informed Consent

From all participants in the clinical trial, informed consent will be obtained prior to inclusion into the study. Informed consent forms will be written to be easily understood by lay persons, enabling them to understand the aims, procedures and potential risks of participation. Special attention will be paid to radiation safety and any risks associated with magnetic resonance imaging and the pharmacological intervention. Informed Consent forms will specifically address the fact that within the clinical trial an approved drug (Alteplase) will be used outside the approval criteria. Informed Consent forms will also address the possibility of patients being treated with placebo. An experienced physician will be present during recruitment of patients to ensure that all participants are competent to understand the aims, procedures and potential risks. We expect that a number of patients will not be able to give informed consent due to stroke symptoms (e.g. aphasia). In these cases, if available, legal representatives, next of kin, independent physicians, or investigators may give informed consent and enrol a patient according to the patient's presumed will conforming to European and national law and depending on the national and local ethics committee approval. Specific consent forms will be used in these cases. Each national coordinating centre will provide information on the trial and Informed Consent forms in their language. The Informed Consent forms will include detailed information about the contraindications for and potential risks of MRI. Special attention will be paid to exclude patients with contraindications for MRI (e.g. cardiac pacemakers) and to provide an adequate monitoring of vital signs during the scanning period. The European patient's organisation Stroke Alliance For Europe (SAFE) and national patient organisations will be involved in the wording of these forms in order to assure that they contain all necessary information and are at the same time understandable to patients.

19.4. Confidentiality

Personal data will be processed in accordance with the EU's Data Protection Directive (Directive 95/46/EC) and regulation (No) EC 45/2001, the relevant national and international legislation, and good practice. Data will only be processed for the trials purposes. Person-identifiable data will not leave the unit from which they originated, and keys to identification numbers will be held confidentially within the respective clinical units. Throughout the trial all individual patients' data will be linked to the e-CRF via a unique identification number (i.e. subject number). Individual patient's medical information will be recorded and transferred to the sponsor only in anonymized form.

The clinical monitors may inspect source data in order to ensure the accuracy of the data recorded in the e-CRF.

19.5. Liability and Insurance

The study sponsor (UKE) provides an appropriate insurance for patients in the event of any trial related damage in accordance with applicable European and national laws. A certificate of insurance will be provided to the investigator in countries in which this document is required.

20. National specifications

The following specifications of the protocol apply to the corresponding countries.

20.1. France

Sample size (added):

It is planned to screen 360 patients and to randomize 120 patients in France.

Ethical and regulatory considerations (add):

Commission Nationale de l'Informatique et des Libertés (CNIL) – This study does not conduct any research in genetics. Neither the CRF nor any other study documents will allow for revealing the complete identity of the patients. So, this study enters in the reference methodology MR001 law. This study will have a simplified declaration with the CNIL.

Regulatory authority (add):

Comité de Protection des Personnes Sud Est IV – Centre Léon Bérard, 28 rue Laënnec, 69373 LYON cedex 08 and the Agence Française de Sécurité Sanitaire des Produits de Santé for France

The trial will be conducted according to the principles laid down (add):

La législation française : la loi 2004-806 du 09 août 2004 relative à la politique de santé publique et au décret d'application n° 2006-477 du 26 avril 2006, aux lignes directrices des Bonnes Pratiques Cliniques Française et Européenne, aux recommandations des ICH (ICH Topic E6 Guideline for Good Clinical Practice), à la loi relative à l'informatique, aux fichiers et aux libertés (loi 2004-801 du 6 août 2004).

Confidentiality (add)

Personal data will be processed in accordance with the law relative à l'informatique, aux fichiers et aux libertés (loi 2004-801 du 6 août 2004) for the France.

21. Administrative procedures

21.1. Curriculum vitae

An updated copy of the curriculum vitae of each investigator and co-investigator will be provided to the responsible national coordinating centre prior to the beginning of the study.

21.2. Secrecy agreement

The investigator shall consider as confidential and shall take all necessary measures to ensure that there is no breach of confidentiality in respect of all information accumulated, acquired or deduced in the course of the trial, other than that information to be disclosed by law.

21.3. Ownership of data and use of the study results

All goods, materials, information (oral or written) and unpublished documentation provided to the investigators (or any company acting on their behalf), inclusive of this study, and the patient case report forms are the exclusive property of the sponsor. The sponsor has the ownership of all data and results collected during this study. In consequence the sponsor reserves the right to use the data of the present study, either in the form of case report forms (or copies of these), or in the form of a report, with or without comments and with or without analysis, in order to submit them to the health authorities of any country.

21.4. Company audits and inspections by regulatory agencies

For the purpose of ensuring compliance with good clinical practice and regulatory agency guidelines it may be necessary to conduct a site audit or an inspection.

By signing this study, the investigator agrees to allow the sponsor and its representative, and drug regulatory agencies to have direct access to his study records for review. These personnel, bound by professional secrecy, will not disclose any personal identity or personal medical information.

These audits involve review of source documents supporting the adequacy and accuracy of data gathered in CRF, review of documentation required to be maintained, and checks on drug accountability.

The sponsor will in all cases help the investigator prepare for an inspection by any regulatory agency.

21.5. Study amendments

It is specified that the appendices attached to this study and referred to in the main text of this study, form an integral part of the study. No changes or amendments to this study may be made by the investigator or by the sponsor after the study has been agreed to and signed by both parties unless such change(s) or amendment(s) have been fully discussed and agreed upon by the investigator and the sponsor. Any change agreed upon will be recorded in writing, the written amendment will be signed by the investigator and by the sponsor and the signed amendment will be appended to this study.

If the change or deviation increases risk to the study population, or adversely affects the validity of the clinical investigation or the subject's rights, full approval / advice must be obtained prior to implementation. For changes that do not involve increased risk or affect the validity of the investigation or the subject's rights, approval / advice may be obtained by expedited review, where applicable.

In some instances, an amendment may require a change to a consent form. The investigator must receive approval / advice of the revised consent form prior to implementation of the change. In addition, changes to the case report forms, if required, will be incorporated in the amendment.

22. Archiving

The investigator will archive all study related documents and trial data in a safe and secure location according to ICH-GCP guidelines and take measures to prevent accidental or premature destruction of these documents. All documents must be held easily available if needed, e.g. in case of audit or inspection. No trial related data should be destroyed without the sponsor’s agreement. Any source data will be archived according to the archiving regulations of the investigational sites following local and national regulations.

For trials performed in the European Community, the investigator is required to arrange for the retention of the patient identification codes for at least 15 years after the completion or discontinuation of the trial.

Any investigational site will notify the sponsor before destroying any data or records.

23. Study Report and Publications

The results of the trial will be reported to the European and National regulatory authorities and ethics committees. The sponsor will provide an annual safety report as well as a final report.

Based on the analysis pre-specified in the clinical trial protocol the results of the trial will be published in the appropriate scientific media.

By signing the clinical trial protocol the investigator agrees that the results of the clinical trial may be used for publication. The investigator also agrees that he is not permitted to publish any data related to the trial independent of the sponsor.

The trial will be registered at ClinicalTrials.gov.

24. References

Adams HP, Jr., Effron MB, Torner J, Davalos A, Frayne J, Teal P, et al. Emergency administration of abciximab for treatment of patients with acute ischemic stroke: results of an international phase III trial: Abciximab in Emergency Treatment of Stroke Trial (AbESTT-II). Stroke 2008a; 39: 87-99.

Adams HP, Jr., Leclerc JR, Bluhmki E, Clarke W, Hansen MD, Hacke W. Measuring outcomes as a function of baseline severity of ischemic stroke. Cerebrovasc Dis 2004; 18: 124-9.

Adams HP, Jr., Leira EC, Torner JC, Barnathan E, Padgett L, Effron MB, et al. Treating Patients With 'Wake-Up' Stroke. The Experience of the AbESTT-II Trial. Stroke 2008b.

Ahmed N, Wahlgren N, Grond M, Hennerici M, Lees KR, Mikulik R, et al. Implementation and outcome of thrombolysis with alteplase 3-4.5 h after an acute stroke: an updated analysis from SITS-ISTR. Lancet Neurol 2010; 9: 866-74.

Allender S, Scarborough P, Viv P, Rayner M, Leal JS, Luengo-Fernandez R, et al. European cardiovascular disease statistics: European Heart Network, 2008.

Aoki J, Kimura K, Iguchi Y, Shibazaki K, Sakai K, Iwanaga T. FLAIR can estimate the onset time in acute ischemic stroke patients. J Neurol Sci 2010; 293: 39-44.

Barber PA, Zhang J, Demchuk AM, Hill MD, Buchan AM. Why are stroke patients excluded from TPA therapy? An analysis of patient eligibility. Neurology 2001; 56: 1015-20.

Barreto AD, Martin-Schild S, Hallevi H, Morales MM, Abraham AT, Gonzales NR, et al. Thrombolytic Therapy for Patients Who Wake-Up With Stroke. Stroke 2009.

Brant-Zawadzki M, Atkinson D, Detrick M, Bradley WG, Scidmore G. Fluid-attenuated inversion recovery (FLAIR) for assessment of cerebral infarction. Initial clinical experience in 50 patients. Stroke 1996; 27: 1187-91.

Breslow NE, Day NE. Statistical methods in cancer research. Volume I - The analysis of case-control studies. IARC Sci Publ 1980: 5-338.

Breuer L, Schellinger PD, Huttner HB, Halwachs R, Engelhorn T, Doerfler A, et al. Feasibility and safety of magnetic resonance imaging-based thrombolysis in patients with stroke on awakening: initial single-centre experience. Int J Stroke 2010; 5: 68-73.

Breuer L, Blinzler C, Huttner HB, Kiphuth IC, Schwab S, Kohrmann M. Off-label thrombolysis for acute ischemic stroke: rate, clinical outcome and safety are influenced by the definition of 'minor stroke'. Cerebrovasc Dis 2011; 32: 177-85.

Cannon CP, McCabe CH, Stone PH, Schactman M, Thompson B, Theroux P, et al. Circadian variation in the onset of unstable angina and non-Q-wave acute myocardial infarction (the TIMI III Registry and TIMI IIIB). Am J Cardiol 1997; 79: 253-8.

Chernyshev OY, Martin-Schild S, Albright KC, Barreto A, Misra V, Acosta I, et al. Safety of tPA in stroke mimics and neuroimaging-negative cerebral ischemia. Neurology 2011; 74: 1340-5.

Chaturvedi S, Adams HP, Jr., Woolson RF. Circadian variation in ischemic stroke subtypes. Stroke 1999; 30: 1792-5.

Cho AH, Sohn SI, Han MK, Lee DH, Kim JS, Choi CG, et al. Safety and efficacy of MRI-based thrombolysis in unclear-onset stroke. A preliminary report. Cerebrovasc Dis 2008; 25: 572-9.

Davis SM, Donnan GA, Parsons MW, Levi C, Butcher KS, Peeters A, et al. Effects of alteplase beyond 3 h after stroke in the Echoplanar Imaging Thrombolytic Evaluation Trial (EPITHET): a placebo-controlled randomised trial. Lancet Neurol 2008; 7: 299-309.

Del Zoppo GJ, Saver JL, Jauch EC, Adams HP, Jr. Expansion of the time window for treatment of acute ischemic stroke with intravenous tissue plasminogen activator: a science advisory from the American Heart Association/American Stroke Association. Stroke 2009; 40: 2945-8.

De Keyser J, Gdovinova Z, Uyttenboogaart M, Vroomen PC, Luijckx GJ. Intravenous alteplase for stroke: beyond the guidelines and in particular clinical situations. Stroke 2007; 38: 2612-8.

Ebinger M, Galinovic I, Rozanski M, Brunecker P, Endres M, Fiebach JB. Fluid-attenuated inversion recovery evolution within 12 hours from stroke onset: a reliable tissue clock? Stroke 2010a; 41: 250-5.

Ebinger M, Ostwaldt AC, Galinovic I, Rozanski M, Brunecker P, Nolte CH, et al. Clinical and radiological courses do not differ between fluid-attenuated inversion recovery-positive and negative patients with stroke after thrombolysis. Stroke 2010b; 41: 1823-5.

Ebinger M, Christensen S, De Silva DA, Parsons MW, Levi CR, Butcher KS, et al. Expediting MRI-based proof-of-concept stroke trials using an earlier imaging end point. Stroke 2009; 40: 1353-8.

ESO. Guidelines for management of ischaemic stroke and transient ischaemic attack 2008. Cerebrovasc Dis 2008; 25: 457-507.

Fachinformation Actilyse®: Boehringer Ingelheim Pharma GmbH Co.KG, 2014.

Fink JN, Kumar S, Horkan C, Linfante I, Selim MH, Caplan LR, et al. The stroke patient who woke up: clinical and radiological features, including diffusion and perfusion MRI. Stroke 2002; 33: 988-93.

Furlan AJ, Eyding D, Albers GW, Al-Rawi Y, Lees KR, Rowley HA, et al. Dose Escalation of Desmoteplase for Acute Ischemic Stroke (DEDAS): evidence of safety and efficacy 3 to 9 hours after stroke onset. Stroke 2006; 37: 1227-31.

Gauvrit JY, Leclerc X, Girot M, Cordonnier C, Sotoares G, Henon H, et al. Fluid-attenuated inversion recovery (FLAIR) sequences for the assessment of acute stroke: inter observer and inter technique reproducibility. J Neurol 2006; 253: 631-5.

Geller NL, Pocock SJ. Interim analyses in randomized clinical trials: ramifications and guidelines for practitioners. Biometrics 1987; 43: 213-23.

Go KG. The normal and pathological physiology of brain water. Adv Tech Stand Neurosurg 1997; 23: 47-142.

Hacke W, Albers G, Al-Rawi Y, Bogousslavsky J, Davalos A, Eliasziw M, et al. The Desmoteplase in Acute Ischemic Stroke Trial (DIAS): a phase II MRI-based 9-hour window acute stroke thrombolysis trial with intravenous desmoteplase. Stroke 2005; 36: 66-73.

Hacke W, Furlan AJ, Al-Rawi Y, Davalos A, Fiebach JB, Gruber F, et al. Intravenous desmoteplase in patients with acute ischaemic stroke selected by MRI perfusion-diffusion weighted imaging or perfusion CT (DIAS-2): a prospective, randomised, double-blind, placebo-controlled study. Lancet Neurol 2009; 8: 141-50.

Hacke W, Kaste M, Bluhmki E, Brozman M, Davalos A, Guidetti D, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med 2008; 359: 1317-29.

Hacke W, Kaste M, Fieschi C, Toni D, Lesaffre E, von Kummer R, et al. Intravenous thrombolysis with recombinant tissue plasminogen activator for acute hemispheric stroke. The European Cooperative Acute Stroke Study (ECASS). Jama 1995; 274: 1017-25.

Hacke W, Kaste M, Fieschi C, von Kummer R, Davalos A, Meier D, et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). Second European-Australasian Acute Stroke Study Investigators. Lancet 1998; 352: 1245-51.

Hellier KD, Hampton JL, Guadagno JV, Higgins NP, Antoun NM, Day DJ, et al. Perfusion CT helps decision making for thrombolysis when there is no clear time of onset. J Neurol Neurosurg Psychiatry 2006; 77: 417-9.

Hjort N, Butcher K, Davis SM, Kidwell CS, Koroshetz WJ, Rother J, et al. Magnetic resonance imaging criteria for thrombolysis in acute cerebral infarct. Stroke 2005; 36: 388-97.

Hoehn-Berlage M, Eis M, Back T, Kohno K, Yamashita K. Changes of relaxation times (T1, T2) and apparent diffusion coefficient after permanent middle cerebral artery occlusion in the rat: temporal evolution, regional extent, and comparison with histology. Magn Reson Med 1995; 34: 824-34.

Horikawa Y, Naruse S, Tanaka C, Hirakawa K, Nishikawa H. Proton NMR relaxation times in ischemic brain edema. Stroke 1986; 17: 1149-52.

Iosif C, Oppenheim C, Trystram D, Domigo V, Meder JF. MR imaging-based decision in thrombolytic therapy for stroke on awakening: report of 2 cases. AJNR Am J Neuroradiol 2008; 29: 1314-6.

Jimenez-Conde J, Ois A, Rodriguez-Campello A, Gomis M, Roquer J. Does sleep protect against ischemic stroke? Less frequent ischemic strokes but more severe ones. J Neurol 2007; 254: 782-8.

Kato H, Kogure K, Ohtomo H, Tobita M, Matsui S, Yamamoto E, et al. Correlations between proton nuclear magnetic resonance imaging and retrospective histochemical images in experimental cerebral infarction. J Cereb Blood Flow Metab 1985; 5: 267-74.

Lees KR, Asplund K, Carolei A, Davis SM, Diener HC, Kaste M, et al. Glycine antagonist (gavestinel) in neuroprotection (GAIN International) in patients with acute stroke: a randomised controlled trial. GAIN International Investigators. Lancet 2000; 355: 1949-54.

Lees KR, Bluhmki E, von Kummer R, Brott TG, Toni D, Grotta JC, et al. Time to treatment with intravenous alteplase and outcome in stroke: an updated pooled analysis of ECASS, ATLANTIS, NINDS, and EPITHET trials. Lancet 2010; 375: 1695-703.

Levy RM, Mano I, Brito A, Hosobuchi Y. NMR imaging of acute experimental cerebral ischemia: time course and pharmacologic manipulations. AJNR Am J Neuroradiol 1983; 4: 238-41.

Lutsep HL, Albers GW, DeCrespigny A, Kamat GN, Marks MP, Moseley ME. Clinical utility of diffusion-weighted magnetic resonance imaging in the assessment of ischemic stroke. Ann Neurol 1997; 41: 574-80.

Mintorovitch J, Moseley ME, Chileuitt L, Shimizu H, Cohen Y, Weinstein PR. Comparison of diffusion- and T2-weighted MRI for the early detection of cerebral ischemia and reperfusion in rats. Magn Reson Med 1991; 18: 39-50.

Mishra NK, Ahmed N, Davalos A, Iversen HK, Melo T, Soinne L, et al. Thrombolysis outcomes in acute ischemic stroke patients with prior stroke and diabetes mellitus. Neurology 2011; 77: 1866-72.

Moseley ME, Kucharczyk J, Mintorovitch J, Cohen Y, Kurhanewicz J, Derugin N, et al. Diffusion-weighted MR imaging of acute stroke: correlation with T2-weighted and magnetic susceptibility-enhanced MR imaging in cats. AJNR Am J Neuroradiol 1990; 11: 423-9.

Muir KW, Buchan A, von Kummer R, Rother J, Baron JC. Imaging of acute stroke. Lancet Neurol 2006; 5: 755-68.

Muller JE, Stone PH, Turi ZG, Rutherford JD, Czeisler CA, Parker C, et al. Circadian variation in the frequency of onset of acute myocardial infarction. N Engl J Med 1985; 313: 1315-22.

Nadeau JO, Fang J, Kapral MK, Silver FL, Hill MD. Outcome after stroke upon awakening. Can J Neurol Sci 2005; 32: 232-6.

Nedeltchev K, Schwegler B, Haefeli T, Brekenfeld C, Gralla J, Fischer U, et al. Outcome of stroke with mild or rapidly improving symptoms. Stroke 2007; 38: 2531-5.

NINDS (The National Institute of Neurological Disorders and Stroke rt-PA Stroke) Study Group. Tissue plasminogen activator for acute ischemic stroke. N Engl J Med 1995; 333: 1581-7.

Noguchi K, Ogawa T, Inugami A, Fujita H, Hatazawa J, Shimosegawa E, et al. MRI of acute cerebral infarction: a comparison of FLAIR and T2-weighted fast spin-echo imaging. Neuroradiology 1997; 39: 406-10.

OECD. Health at a Glance: OECD Indicators 2003. Paris: OECD Publishing, 2003.

OECD. Health at a Glance: Europe 2010. Paris: OECD Publishing, 2010.

Ostergaard L, Sorensen AG, Kwong KK, Weisskoff RM, Gyldensted C, Rosen BR. High resolution measurement of cerebral blood flow using intravascular tracer bolus passages. Part II: Experimental comparison and preliminary results. Magn Reson Med 1996; 36: 726-36.

Perkins CJ, Kahya E, Roque CT, Roche PE, Newman GC. Fluid-attenuated inversion recovery and diffusion- and perfusion-weighted MRI abnormalities in 117 consecutive patients with stroke symptoms. Stroke 2001; 32: 2774-81.

Ricci PE, Burdette JH, Elster AD, Reboussin DM. A comparison of fast spin-echo, fluid-attenuated inversion-recovery, and diffusion-weighted MR imaging in the first 10 days after cerebral infarction. AJNR Am J Neuroradiol 1999; 20: 1535-42.

Saver JL. Number needed to treat estimates incorporating effects over the entire range of clinical outcomes: novel derivation method and application to thrombolytic therapy for acute stroke. Arch Neurol 2004; 61: 1066-70.

Saver JL. Novel end point analytic techniques and interpreting shifts across the entire range of outcome scales in acute stroke trials. Stroke 2007; 38: 3055-62.

Schlaug G, Siewert B, Benfield A, Edelman RR, Warach S. Time course of the apparent diffusion coefficient (ADC) abnormality in human stroke. Neurology 1997; 49: 113-9.

Schuier FJ, Hossmann KA. Experimental brain infarcts in cats. II. Ischemic brain edema. Stroke 1980; 11: 593-601.

Serena J, Davalos A, Segura T, Mostacero E, Castillo J. Stroke on awakening: looking for a more rational management. Cerebrovasc Dis 2003; 16: 128-33.

Silva GS, Lima FO, Camargo EC, Smith WS, Singhal AB, Greer DM, et al. Wake-up stroke: clinical and neuroimaging characteristics. Cerebrovasc Dis 2010; 29: 336-42.

Singer OC, Humpich MC, Fiehler J, Albers GW, Lansberg MG, Kastrup A, et al. Risk for symptomatic intracerebral hemorrhage after thrombolysis assessed by diffusion-weighted magnetic resonance imaging. Ann Neurol 2008; 63: 52-60.

Sorensen AG, Buonanno FS, Gonzalez RG, Schwamm LH, Lev MH, Huang-Hellinger FR, et al. Hyperacute stroke: evaluation with combined multisection diffusion-weighted and hemodynamically weighted echo-planar MR imaging. Radiology 1996; 199: 391-401.

Stanley K. Design of randomized controlled trials. Circulation 2007; 115: 1164-9.

Tan SB, Machin D. Bayesian two-stage designs for phase II clinical trials. Stat Med 2002; 21: 1991-2012.

Thomalla G, Cheng B, Ebinger M, Hao Q, Tourdias T, Wu O, et al. DWI-FLAIR mismatch for the identification of patients with acute ischaemic stroke within 4.5 h of symptom onset (PRE-FLAIR): a multicentre observational study. Lancet Neurol 2011; 10:978-86.

Thomalla G, Gerloff C. We are on the clock: MRI as a surrogate marker of lesion age in acute ischemic stroke. Stroke 2010; 41: 197-8.

Thomalla G, Rossbach P, Rosenkranz M, Siemonsen S, Krutzelmann A, Fiehler J, et al. Negative fluid-attenuated inversion recovery imaging identifies acute ischemic stroke at 3 hours or less. Ann Neurol 2009; 65: 724-32.

Tilley BC, Marler J, Geller NL, Lu M, Legler J, Brott T, et al. Use of a global test for multiple outcomes in stroke trials with application to the National Institute of Neurological Disorders and Stroke t-PA Stroke Trial. Stroke 1996; 27: 2136-42.

Todo K, Moriwaki H, Saito K, Tanaka M, Oe H, Naritomi H. Early CT findings in unknown-onset and wake-up strokes. Cerebrovasc Dis 2006; 21: 367-71.

Tsivgoulis G, Alexandrov AV, Chang J, Sharma VK, Hoover SL, Lao AY, et al. Safety and outcomes of intravenous thrombolysis in stroke mimics: a 6-year, single-care center study and a pooled analysis of reported series. Stroke 2011; 42: 1771-4.

Venkatesan R, Lin W, Gurleyik K, He YY, Paczynski RP, Powers WJ, et al. Absolute measurements of water content using magnetic resonance imaging: preliminary findings in an in vivo focal ischemic rat model. Magn Reson Med 2000; 43: 146-50.

Wahlgren N, Ahmed N, Davalos A, Ford GA, Grond M, Hacke W, et al. Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST): an observational study. Lancet 2007; 369: 275-82.

Wardlaw JM, Murray V, Berge E, Del Zoppo GJ. Thrombolysis for acute ischaemic stroke. Cochrane Database Syst Rev 2009: CD000213.

Watanabe O, West CR, Bremer A. Experimental regional cerebral ischemia in the middle cerebral artery territory in primates. Part 2: Effects on brain water and electrolytes in the early phase of MCA stroke. Stroke 1977; 8: 71-6.

Weimar C, Konig IR, Kraywinkel K, Ziegler A, Diener HC. Age and National Institutes of Health Stroke Scale Score within 6 hours after onset are accurate predictors of outcome after cerebral ischemia: development and external validation of prognostic models. Stroke 2004; 35: 158-62.

WHO. The Atlas of Heart Disease and Stroke In: Mackay J and Mensah GA, editors. Geneva: World Health Organisation, 2004.

25. Approval and Signatures

Sponsor Signature Page

This protocol has been approved by the University Medical Center Hamburg-Eppendorf.

05/06/2015

Sponsor Signature

Date

Universität Hamburg
Universitätsklinikum Hamburg-Eppendorf
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Dekan
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Prof. Dr. Dr. Uwe Koch-Gromus (Dean)

Sponsor Name

Coordinating Investigator Signature Page

I hereby confirm that I have acknowledged the protocol and agree to conduct the study in compliance with the protocol.

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09.06.2015

Coordinating Investigator Signature

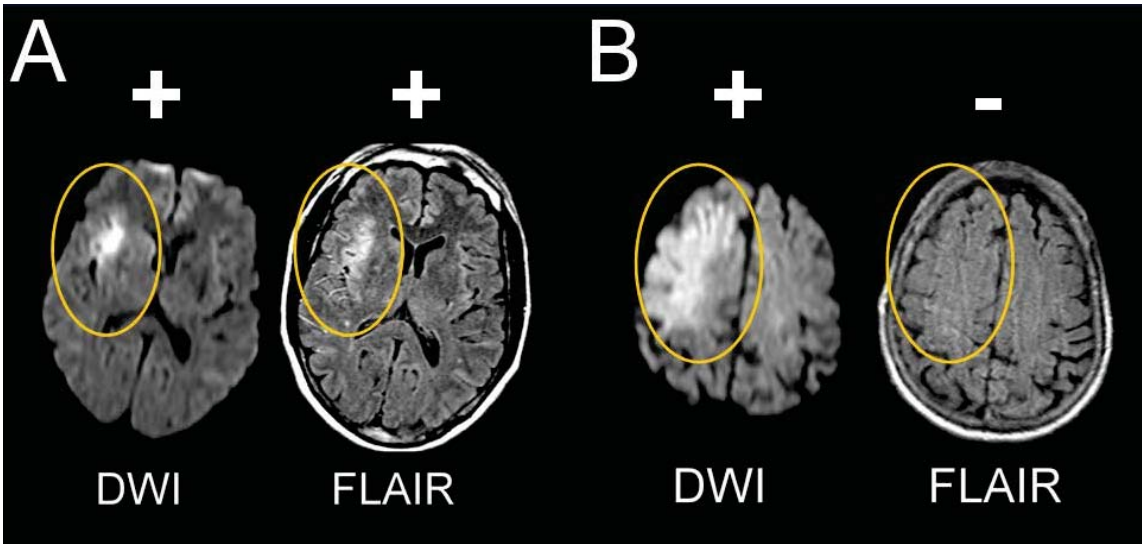
Date

PD Dr. Götz Thomalla

Coordinating Investigator Name

26. Appendices

26.1. Appendix A: DWI-FLAIR examples



A - Acute ischemic lesion clearly visible (+) both on DWI and FLAIR: no "DWI-FLAIR mismatch"; imaging inclusion criteria not met.

B - Acute ischemic lesion clearly visible (+) on DWI but no hyperintensity traceable on FLAIR (-): "DWI-FLAIR mismatch", acute ischemic lesion likely to be less than 4.5 hours of age; imaging inclusion criteria fulfilled.

26.2. Appendix B: NIHSS

NIHSS page 1

NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

Time: ___ ___:___ ___ [ ]am [ ]pm

Person Administering Scale _____

Administer stroke scale items in the order listed. Record performance in each category after each subscale exam. Do not go back and change scores. Follow directions provided for each exam technique. Scores should reflect what the patient does, not what the clinician thinks the patient can do. The clinician should record answers while administering the exam and work quickly. Except where indicated, the patient should not be coached (i.e., repeated requests to patient to make a special effort).

Instructions	Scale Definition	Score
1a. Level of Consciousness: The investigator must choose a response if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation.	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. LOC Questions: The patient is asked the month and his/her age. The answer must be correct - there is no partial credit for being close. Aphasic and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier, or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues.	0 = **Answers** both questions correctly. 1 = **Answers** one question correctly. 2 = **Answers** neither question correctly.	_____
1c. LOC Commands: The patient is asked to open and close the eyes and then to grip and release the non-paretic hand. Substitute another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to him or her (pantomime), and the result scored (i.e., follows none, one or two commands). Patients with trauma, amputation, or other physical impediments should be given suitable one-step commands. Only the first attempt is scored.	0 = **Performs** both tasks correctly. 1 = **Performs** one task correctly. 2 = **Performs** neither task correctly.	_____
2. Best Gaze: Only horizontal eye movements will be tested. Voluntary or reflexive (oculocephalic) eye movements will be scored, but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an isolated peripheral nerve paresis (CN III, IV or VI), score a 1. Gaze is testable in all aphasic patients. Patients with ocular trauma, bandages, pre-existing blindness, or other disorder of visual acuity or fields should be tested with reflexive movements, and a choice made by the investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence of a partial gaze palsy.	0 = **Normal.** 1 = **Partial gaze palsy;** gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present. 2 = **Forced deviation,** or total gaze paresis not overcome by the oculocephalic maneuver.	_____

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NIHSS page 2

NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

3. Visual: Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat, as appropriate. Patients may be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia, is found. If patient is blind from any cause, score 3. Double simultaneous stimulation is performed at this point. If there is extinction, patient receives a 1, and the results are used to respond to item 11.	0 = **No visual loss.** 1 = **Partial hemianopia.** 2 = **Complete hemianopia.** 3 = **Bilateral hemianopia** (blind including cortical blindness).	_____
4. Facial Palsy: Ask – or use pantomime to encourage – the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barriers obscure the face, these should be removed to the extent possible.	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. Motor Arm: The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine). Drift is scored if the arm falls before 10 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in the case of amputation or joint fusion at the shoulder, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	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, explain: _____ **5a. Left Arm** **5b. Right Arm**	_____ _____
6. Motor Leg: The limb is placed in the appropriate position: hold the leg at 30 degrees (always tested supine). Drift is scored if the leg falls before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic leg. Only in the case of amputation or joint fusion at the hip, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	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 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, explain: _____ **6a. Left Leg** **6b. Right Leg**	_____

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NIHSS page 3

N I H STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

7. Limb Ataxia: This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual defect, ensure testing is done in intact visual field. The finger-nose-finger and heel-shin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice. In case of blindness, test by having the patient touch nose from extended arm position.	0 = **Absent.** 1 = **Present in one limb.** 2 = **Present in two limbs.** UN = **Amputation** or joint fusion, explain: _____	_____
8. Sensory: Sensation or grimace to pinprick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas (arms [not hands], legs, trunk, face) as needed to accurately check for hemisensory loss. A score of 2, "severe or total sensory loss," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will, therefore, probably score 1 or 0. The patient with brainstem stroke who has bilateral loss of sensation is scored 2. If the patient does not respond and is quadriplegic, score 2. Patients in a coma (item 1a=3) are automatically given a 2 on this item.	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 to total sensory loss;** patient is not aware of being touched in the face, arm, and leg.	_____
9. Best Language: A great deal of information about comprehension will be obtained during the preceding sections of the examination. For this scale item, the patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet and to read from the attached list of sentences. Comprehension is judged from responses here, as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to identify objects placed in the hand, repeat, and produce speech. The intubated patient should be asked to write. The patient in a coma (item 1a=3) will automatically score 3 on this item. The examiner must choose a score for the patient with stupor or limited cooperation, but a score of 3 should be used only if the patient is mute and follows no one-step commands.	0 = **No aphasia;** normal. 1 = **Mild-to-moderate aphasia;** some obvious 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.	_____
10. Dysarthria: If patient is thought to be normal, an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other physical barriers to producing speech, the examiner should record the score as untestable (UN), and clearly write an explanation for this choice. Do not tell the patient why he or she is being tested.	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, explain:_____	_____

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NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

11. Extinction and Inattention (formerly Neglect): Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosagnosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable.	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.	____

____

____

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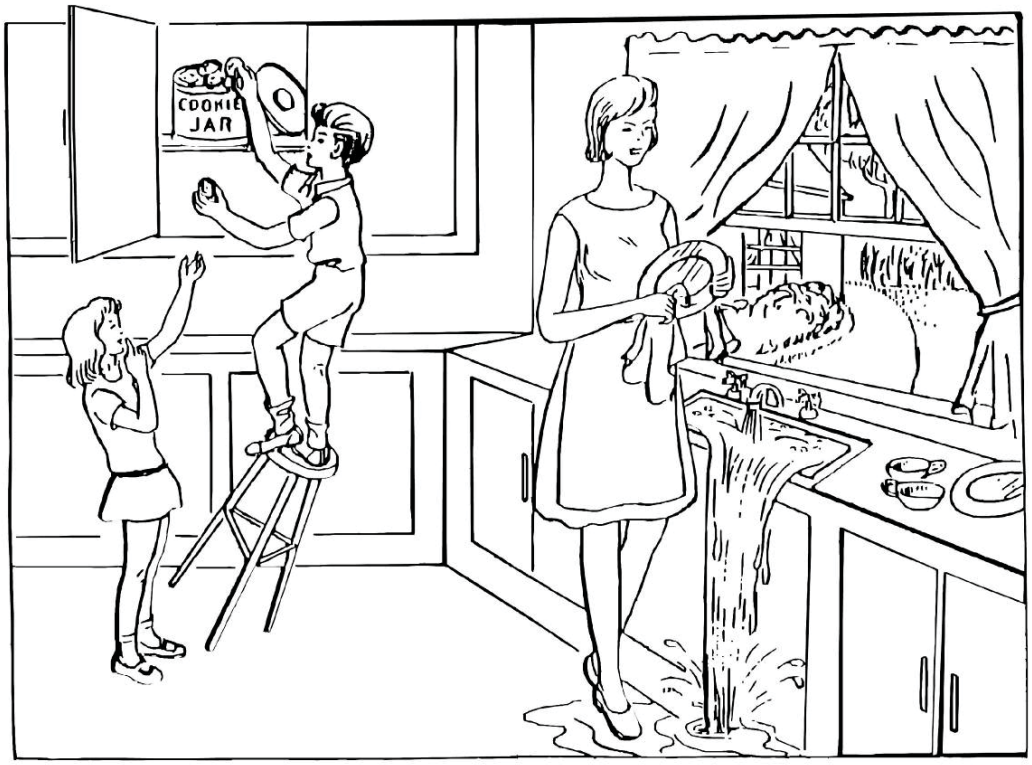


You know how.

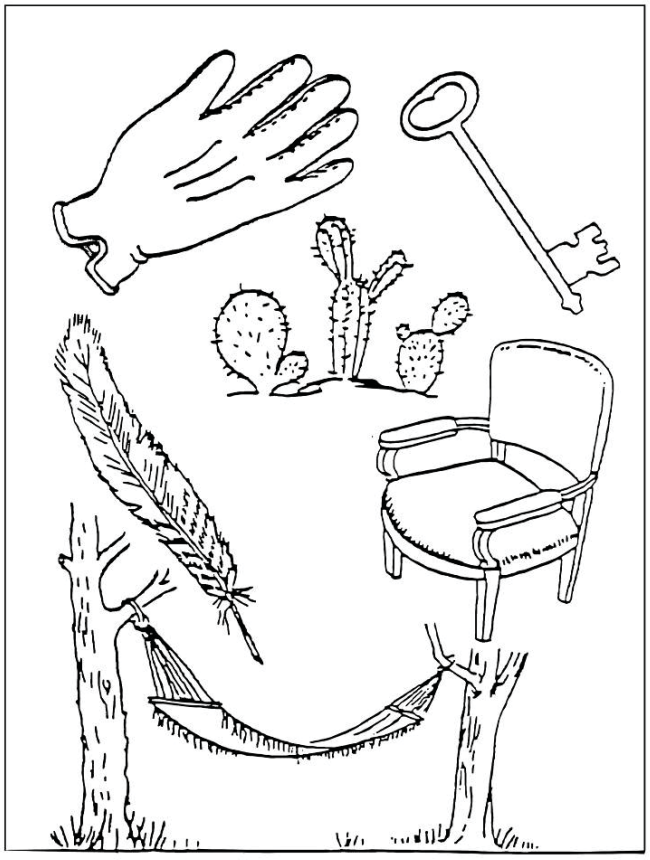
Down to earth.

I got home from work.

Near the table in the dining room.

They heard him speak on the radio last night.

NIHSS page 6



MAMA

TIP – TOP

FIFTY – FIFTY

THANKS

HUCKLEBERRY

BASEBALL PLAYER

26.3. Appendix C: MRS

MODIFIED RANKIN SCALE (MRS)

Patient Name: _____
Rater Name: _____
Date: _____

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

TOTAL (0–6): _____

References

Rankin J. "Cerebral vascular accidents in patients over the age of 60."
Scott Med J 1957;2:200-15

Bonita R, Beaglehole R. "Modification of Rankin Scale: Recovery of motor function after stroke."
***Stroke* 1988 Dec;19(12):1497-1500**

Van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. "Interobserver agreement for the assessment of handicap in stroke patients."
***Stroke* 1988;19(5):604-7**

26.4. Appendix D: BI

BI page 1

THE BARTHEL INDEX

Patient Name: _____
Rater Name: _____
Date: _____

Activity	Score
FEEDING 0 = unable 5 = needs help cutting, spreading butter, etc., or requires modified diet 10 = independent	_____
BATHING 0 = dependent 5 = independent (or in shower)	_____
GROOMING 0 = needs to help with personal care 5 = independent face/hair/teeth/shaving (implements provided)	_____
DRESSING 0 = dependent 5 = needs help but can do about half unaided 10 = independent (including buttons, zips, laces, etc.)	_____
BOWELS 0 = incontinent (or needs to be given enemas) 5 = occasional accident 10 = continent	_____
BLADDER 0 = incontinent, or catheterized and unable to manage alone 5 = occasional accident 10 = continent	_____
TOILET USE 0 = dependent 5 = needs some help, but can do something alone 10 = independent (on and off, dressing, wiping)	_____
TRANSFERS (BED TO CHAIR AND BACK) 0 = unable, no sitting balance 5 = major help (one or two people, physical), can sit 10 = minor help (verbal or physical) 15 = independent	_____
MOBILITY (ON LEVEL SURFACES) 0 = immobile or < 50 yards 5 = wheelchair independent, including corners, > 50 yards 10 = walks with help of one person (verbal or physical) > 50 yards 15 = independent (but may use any aid; for example, stick) > 50 yards	_____
STAIRS 0 = unable 5 = needs help (verbal, physical, carrying aid) 10 = independent	_____
TOTAL (0–100):	_____

Provided by the Internet Stroke Center — www.strokecenter.org

BI page 2

The Barthel ADL Index: Guidelines

1. The index should be used as a record of what a patient does, not as a record of what a patient could do.
2. The main aim is to establish degree of independence from any help, physical or verbal, however minor and for whatever reason.
3. The need for supervision renders the patient not independent.
4. A patient's performance should be established using the best available evidence. Asking the patient, friends/relatives and nurses are the usual sources, but direct observation and common sense are also important. However direct testing is not needed.
5. Usually the patient's performance over the preceding 24-48 hours is important, but occasionally longer periods will be relevant.
6. Middle categories imply that the patient supplies over 50 per cent of the effort.
7. Use of aids to be independent is allowed.

References

Mahoney FI, Barthel D. "Functional evaluation: the Barthel Index."
Maryland State Medical Journal 1965;14:56-61. Used with permission.

Loewen SC, Anderson BA. "Predictors of stroke outcome using objective measurement scales."
***Stroke.* 1990;21:78-81.**

Gresham GE, Phillips TF, Labi ML. "ADL status in stroke: relative merits of three standard indexes."
***Arch Phys Med Rehabil.* 1980;61:355-358.**

Collin C, Wade DT, Davies S, Horne V. "The Barthel ADL Index: a reliability study."
***Int Disability Study.*1988;10:61-63.**

Copyright Information

26.5. Appendix E: GOS

GLASGOW OUTCOME SCALE

Patient Name: _____
Rater Name: _____
Date: _____

Note: The scale presented here is based on the original article by Jennett and Bond. It has become common practice in clinical trial administration, however, to use a modified version that places the scores in reverse order (i.e., "good recovery" = 1, "moderate disability" =2, etc.).

Score	Description
1	DEATH
2	PERSISTENT VEGETATIVE STATE Patient exhibits no *obvious cortical* function.
3	SEVERE DISABILITY (Conscious but disabled). Patient depends upon others for daily support due to mental or physical disability or both.
4	MODERATE DISABILITY (Disabled but independent). Patient is independent as far as daily life is concerned. The disabilities found include varying degrees of dysphasia, hemiparesis, or ataxia, as well as intellectual and memory deficits and personality changes.
5	GOOD RECOVERY Resumption of normal activities even though there may be minor neurological or psychological deficits.

TOTAL (1–5): _____

References

Jennett B, Bond M. "Assessment of outcome after severe brain damage."
Lancet 1975 Mar 1;1(7905):480-4

26.6. Appendix F: BDI

BDI (English version) page 1

1.
- 0 I do not feel sad.
- 1 I feel sad
- 2 I am sad all the time and I can't snap out of it.
- 3 I am so sad and unhappy that I can't stand it.

2.
- 0 I am not particularly discouraged about the future.
- 1 I feel discouraged about the future.
- 2 I feel I have nothing to look forward to.
- 3 I feel the future is hopeless and that things cannot improve.

3.
- 0 I do not feel like a failure.
- 1 I feel I have failed more than the average person.
- 2 As I look back on my life, all I can see is a lot of failures.
- 3 I feel I am a complete failure as a person.

4.
- 0 I get as much satisfaction out of things as I used to.
- 1 I don't enjoy things the way I used to.
- 2 I don't get real satisfaction out of anything anymore.
- 3 I am dissatisfied or bored with everything.

5.
- 0 I don't feel particularly guilty
- 1 I feel guilty a good part of the time.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6.
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7.
- 0 I don't feel disappointed in myself.
- 1 I am disappointed in myself.
- 2 I am disgusted with myself.
- 3 I hate myself.

8.
- 0 I don't feel I am any worse than anybody else.
- 1 I am critical of myself for my weaknesses or mistakes.
- 2 I blame myself all the time for my faults.
- 3 I blame myself for everything bad that happens.

9.
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10.
- 0 I don't cry any more than usual.
- 1 I cry more now than I used to.
- 2 I cry all the time now.
- 3 I used to be able to cry, but now I can't cry even though I want to.

BDI (English version) page 2

11.
0 I am no more irritated by things than I ever was.
1 I am slightly more irritated now than usual.
2 I am quite annoyed or irritated a good deal of the time.
3 I feel irritated all the time.

12.
0 I have not lost interest in other people.
1 I am less interested in other people than I used to be.
2 I have lost most of my interest in other people.
3 I have lost all of my interest in other people.

13.
0 I make decisions about as well as I ever could.
1 I put off making decisions more than I used to.
2 I have greater difficulty in making decisions more than I used to.
3 I can't make decisions at all anymore.

14.
0 I don't feel that I look any worse than I used to.
1 I am worried that I am looking old or unattractive.
2 I feel there are permanent changes in my appearance that make me look unattractive
3 I believe that I look ugly.

15.
0 I can work about as well as before.
1 It takes an extra effort to get started at doing something.
2 I have to push myself very hard to do anything.
3 I can't do any work at all.

16.
0 I can sleep as well as usual.
1 I don't sleep as well as I used to.
2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
3 I wake up several hours earlier than I used to and cannot get back to sleep.

17.
0 I don't get more tired than usual.
1 I get tired more easily than I used to.
2 I get tired from doing almost anything.
3 I am too tired to do anything.

18.
0 My appetite is no worse than usual.
1 My appetite is not as good as it used to be.
2 My appetite is much worse now.
3 I have no appetite at all anymore.

19.
0 I haven't lost much weight, if any, lately.
1 I have lost more than five pounds.
2 I have lost more than ten pounds.
3 I have lost more than fifteen pounds.

20.
0 I am no more worried about my health than usual.
1 I am worried about physical problems like aches, pains, upset stomach, or constipation.
2 I am very worried about physical problems and it's hard to think of much else.
3 I am so worried about my physical problems that I cannot think of anything else.

21.
0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I have almost no interest in sex.
3 I have lost interest in sex completely.

26.7. Appendix G: EQ-5D

EQ-5D (English version) page 1

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about	❑
I have some problems in walking about	❑
I am confined to bed	❑

Self-Care

I have no problems with self-care	❑
I have some problems washing or dressing myself	❑
I am unable to wash or dress myself	❑

Usual Activities (*e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities	❑
I have some problems with performing my usual activities	❑
I am unable to perform my usual activities	❑

Pain/Discomfort

I have no pain or discomfort	❑
I have moderate pain or discomfort	❑
I have extreme pain or discomfort	❑

Anxiety/Depression

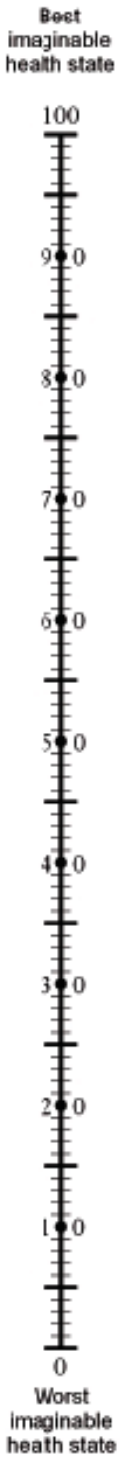
I am not anxious or depressed	❑
I am moderately anxious or depressed	❑
I am extremely anxious or depressed	❑

EQ-5D (English version) page 2

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

Your own health state today



26.8. Appendix H: Questionnaire – use of health care system resources

1. How as the questionnaire completed?
    a. Patient was asked
    b. Caregiver was asked
    c. Information from medical records
    d. Questionnaire was not completed

2. Health status and housing prior to qualifying stroke event
    a. At home
    b. Assisted living
    c. Nursing home
    d. Rehabilitation
    e. Hospital

3. Employment status prior to qualifying stroke event
    a. Working
    b. Not working, scholar, student
    c. Housemaker
    d. Sick leave
    e. Retired

4. Number of nights in hospital for treatment of qualifying stroke event _____

5. Discharged where after treatment of qualifying stroke event
    a. Home
    b. Assisted living
    c. Nursing home
    d. Rehabilitation
    e. Hospital
    f. Died

6. Current health status and housing
    a. At home
    b. Assisted living
    c. Nursing home
    d. Rehabilitation
    e. Hospital
    f. Not applicable (died)

7. Current employment status
    a. Working
    b. Not working, scholar, student
    c. Housemaker
    d. Sick leave
    e. Retired
    f. Not applicable (died)

8. Current rehabilitation activities: has the patient received any rehabilitation services during the past 30 days? (yes/no)
    a. If yes, please specify (average hours per week):
        i. Physiotherapy
        ii. Logopaedia
        iii. Occupational therapy
        iv. Psychological treatment

9. Has there been another referral to a hospital between the treatment of the qualifying stroke event and this follow-up examination (yes/no)
    a. If yes, how often has the patient been referred to hospital since the treatment of the qualifying stroke event?
    b. If applicable, provide reasons (diagnosis) for hospital admissions

26.9. Appendix I: List of National Coordinating Centres

Belgium

Katholieke Universiteit Leuven

Herestraat 49-7003

3000 Leuven

Belgium

Denmark

Aarhus Universitetshospital, Aarhus Sygehus

Norrebrogade 44

8000 Aarhus

Denmark

France

Hospices Civils de Lyon

Boulevard Pinel 59

69677 BRON Cedex

France

Germany

Charité – Universitätsmedizin Berlin

Charitéplatz 1

10117 Berlin

Germany

Great Britain

University of Glasgow

University Avenue

Glasgow G12 8QQ

United Kingdom

Spain

Institut d'Investigacio Biomedica de Girona Doctor Josep Trueta

Avenida de França s/n

17007 Girona

Spain

26.10. Appendix J: List of CRO Names

CRO (Monitoring)

ORION Clinical Services Ltd.
7 Bath Road, Slough, SL1 3AU
United Kingdom

Data Management

bioskin® GmbH
Burchardstrasse 17
20095 Hamburg
Germany

Electronic Case Record Form (eCRF)

Quadratek
Data Solutions Ltd.
Winchfield Lodge
Old Potbridge Road
Winchfield
Hampshire RG27 8BT
United Kingdom

German office:

Quadratek
Data Solutions Ltd.
Albrechtstraße 22
10117 Berlin
Germany