CLINICAL STUDY PROTOCOL

A Randomized, Placebo-controlled, double-blind trial to assess the efficacy and safety of CEREBROLYSIN in the treatment of Post-Stroke Cognitive Decline

Study Code	CODEC
Protocol Number	FSNANO100220
Version	2.0 – amendment 1
Date	May 28th, 2020
Coordinator	Foundation for the Study of Nanoneurosciences and Neuroregeneration (FSNN)

Confidential Information

This protocol has been written in accordance with the ICH-GCP guidelines and the *Declaration of Helsinki* in current versions.

TABLE OF CONTENTS

1. ABBREVIATIONS AND DEFINITIONS

AE	Adverse Event
A(D)R	Adverse (Drug) Reaction
CRB	Cerebrolysin
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Computed tomography
DLPFC	Dorso-lateral pre-frontal cortex
FSNN	Foundation for the Study of Nanoneurosciences and Neuroregeneration
GCP	Good Clinical Practice
ICH	International Conference for Harmonization
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IV	Intra-venous
mL	Milli Liter
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment Scale
MRI	Magnetic Resonance Imaging
PLC	Placebo
SAE	Serious Adverse Event
SAP	Statistical Analyses Plan
SAR	Serious Adverse Reaction
SESAR	Suspected Expected Serious Adverse Reaction

SOP	Standard Operating Procedure
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
WAIS	Wechsler Adult Intelligence Scale
WHO-UMC	World Health Organization-Uppsala Monitoring Center

2. PROTOCOL SUMMARY / SYNOPSIS

Coordinator	FSNN – Foundation for the Study of Nanoneurosciences and Neuroregeneration
Title	A Randomized, Placebo-controlled, double-blind trial to asses the effficacy and safety of CEREBROLYSIN in the treatment of Post-Stroke Cognitive Decline
Study Code	CODEC
Study Location	Institutul RoNeuro Cluj-Napoca
Investigational Medicinal Product	Cerebrolysin Solution for Injection (CRB)
Name of Active Substance	Cerebrolysin Concentrate
Phase	IV
Indication	Acute Ischeamic Stroke
Study Design	Randomized, double-blind, phase IV study
Study Duration	Study start: 05/2020 Study end: 12/2025
Sample Size	• Group 1 – Cerebrolysin N = 145 • Group 2 – Placebo N = 145
Primary Objective	To assess the efficacy of Cerebrolysin versus Placebo upon a battery of co-primary neurocognitive outcome scores at 180 and 360 days after baseline.
Secondary Objectives	To assess the efficacy of Cerebrolysin versus Placebo upon neurological deficit, functional outcome, symptoms of anxiety and depression, drug safety and quality of life 180 and 360 days after baseline.
Primary Variables	The following psychometric tests are included in the primary mutivariate analysis (combined cognitive outcome measures): Stroop test (Stroop) Trail-Making-Test Part A (TMT-A) Digit Span Backwards Task (DS-BW) Verbal Fluency Test – CFL Version (VFT-CFL) Digit Symbol (DS-WPSI) Rey Auditory Verbal Learning Test (RAVLT)
Secondary Variables	Montreal Cognitive Assessment (MOCA)

	NIH Stroke Scale (NIH) Modified Rankin Scale (mRS) Hospital Anxiety and Depression Scale (HADS) EQ-5D-5L
Safety Variables	Adverse Events (AE) Severe Adverse Events (SAE)
Inclusion Criteria	● Diagnosis of stroke, ischemic in origin (TACS or PACS), confirmed by MRI ● Onset of Stroke within 72 hours prior to screening ● NIH Stroke Scale score between 5-15 at inpatient admission ● Pre-stroke mRS of 0 or 1 ● No cognitive impairment prior to stroke with an IQ code score ≤ 3 ● Age between 40 and 80 years, inclusive ● Patient is willing and able to comply with the protocol for the duration of the study
Exclusion Criteria	● Previous symptomatic ischaemic stroke or intracranial hemorrhage not related to the index stroke ● Severe visual or hearing impairment interfering with psychometric test procedures ● Pre-existing and active major neurological disease (eg. Parkinson's Disease, Epilepsy) ● Pre-existing and active major psychiatric disease, such as major depression, schizophrenia, bipolar disease, or dementia ● History of significant alcohol or drug abuse ● Advanced liver, kidney, cardiac, or pulmonary disease ● A terminal medical diagnosis with survival < 1 year ● Pregnancy or lactating ● Any contraindications to Cerebrolysin ● Current enrolment in another therapeutic study ● Dementia due to strategic index stroke ● Major communication deficits with a Goodglass & Kaplan Score lower than 2 ● Aphasia with an NIHSS Item 9 score of ≥ 2 ● Treatment with Cerebrolysin or Neuroprotectants in the last 30 days ● Severe dementia with MMSE Score <12
Visit Schedule	**Visit 1 - Screening Part 1** **Study day -30 – within 72 h after stroke onset** ● Demographic data ● Medical history and risk factors ● IQ code ● NIH Stroke Scale score between 5-15 at inpatient admission ● mRS / premorbid mRS

	● In/Exclusion criteria – Part 1 **Visit 2 - Screening Part 2 & Baseline Study Day 1** ● Informed Consent (signed up to Visit 2 start) ● Goodglass-Kaplan Communication Scale ● MMSE ● In/Exclusion criteria – Part 2 ● Randomization ● Primary Outcome Measures Stroop, TMT-A, DS-BW, VFT-CFL, DS-WPSI, RAVLT ● Secondary Outcome Measures NIH, mRS, HADS, MoCA, EQ-5D-5L ● Safety Information **Visit 3 - Efficacy & Safety Evaluation Study Day 180** ● Primary Outcome Measures Stroop, TMT-A, DS-BW, VFT-CFL, DS-WPSI, RAVLT ● Secondary Outcome Measures NIH, mRS, HADS, MoCA, EQ-5D-5L ● Safety Information **Visit 4 - Efficacy & Safety Evaluation Study Day 360** ● Primary Outcome Measures Stroop, TMT-A, DS-BW, VFT-CFL, DS-WPSI, RAVLT ● Secondary Outcome Measures NIH, mRS, HADS, MoCA, EQ-5D-5L ● Safety Information
Investigational Product	Cerebrolysin Solution fo Injection (CRB): 30 ml diluted with 0.9% saline solution to 250 ml, administered by IV infusion
Reference Product	Placebo: 250 ml 0.9% saline solution administered by IV infusion
Treatment Schedule	Once daily IV infusions of either 30ml CRB or PLC **Treatment Cycle 1** Study day 1 – 10 10 Infusions, once daily **Treatment Cycle 2** Study day 61-70 10 Infusions, once daily **Treatment Cycle 3** Study day 121-130 10 Infusions, once daily

	Treatment Cycle 4 Study day 241-250 10 Infusions, once daily

*All treatment cycles and efficacy evaluations will be performed within a window of ±3 working days.

3. INTRODUCTION

3.1. Background information

Stroke is the second leading cause of death and a major cause of disability worldwide. Its incidence is increasing due to aging population. In addition, more young people are affected by stroke in low- and middle-income countries. Ischemic stroke is characterized by the sudden loss of blood circulation to an area of the brain, resulting in a corresponding loss of neurologic function. Acute ischemic stroke is caused by thrombotic or embolic occlusion of a cerebral artery and is more common than hemorrhagic stroke.

Post-stroke cognitive impairment is a particularly serious consequence of cerebral ischaemia and often inhibits or retards patient rehabilitation. Post-stroke cognitive impairment occurs frequently in patients with stroke. The prevalence of post-stroke cognitive impairment ranges between 20-80%. The risk of post-stroke cognitive impairment is related to both demographic factors like age, education, occupation and vascular factors. The underlying mechanisms PSCI are not fully understood.

However, scientific literature has shown that neuroanatomical lesions caused by stroke on strategic areas such as the hippocampus and white matter lesions, cerebral microbleeds due to the small cerebrovascular diseases and the mixed AD with stroke, alone or in combination, contribute to the pathogenesis of post-stroke cognitive impairment.

Current treatments for PSCI do not improve long-term outcomes for a significant proportion of patients and leave substantially unmet medical needs. Initiatives to address the challenge of post-stroke rehabilitation have included therapies that modify multiple pathogenetic mechanisms and provide protection to neural networks and facilitate their regeneration. Promising biological agents have been tested, but few have so far yielded clinically conclusive evidence, further emphasising the shortage of therapies available to treat this disease. Due to the heterogenous results of clinical studies, additional research is need to determine the efficacy of various therapeutic strategies.

3.2. Study Rationale

Incident stroke alters a patient's cognitive trajectory, and this effect is greater with increasing age and cardioembolic stroke. About one-third of stroke patients have significant cognitive impairment within several months of the event. In addition, silent strokes, experienced by 1 in 10 adults by their early 60s, are harbingers of both future stroke and cognitive dysfunction.

Researchers from the University of Michigan tracked the trajectories of cognitive decline before and after incident stroke in a prospective study of 23,572 participants age 45 years or older without baseline cognitive impairment (Levine et al., 2015). The results show that stroke was associated with acute decline in global cognition, new learning, and verbal memory. Participants with stroke, compared with those without stroke, demonstrated faster

declines in global cognition and executive function, but not in new learning and verbal memory, compared with pre-stroke slopes. These findings suggest that clinicians may have an opportunity to intervene immediately following stroke to prevent accelerated stroke-related cognitive decline.

The neuroprotective adjuvant effect of Cerebrolysin in prevention of postoperative cognitive disfunction has been documented for cardiac (Polushin et al., 2017) and neurosurgical (Matula & Schoeggl, 2000) procedures. Furthermore, the drug has been proven to stabilize cognitive decline and aid in regression of cognitive disorders predicting vascular dementia in a randomized double-blind placebo-controlled trial (Vereshchagin et al., 2001). In a clinical-electrophysiological study of 40 patients, courses of cerebrolysin treatment decreased the severity of memory and attention impairments, improving the overall cognitive status of patients with cerebral vascular insufficiency (Damulin, Koberskaya, & Mkhitaryan, 2008).

The rationale of the study is based on the previously documented neuroprotective characteristics of Cerebrolysin with potential of preventive effects for cognitive decline after stroke.

4. STUDY OBJECTIVES

This study shall assess the efficacy of pharmacological intervention with Cerebrolysin in the prevention of post stroke cognitive decline.

The aim of this trial is to test the hypothesis that patients randomized to Cerebrolysin show improved cognitive outcome measured with a battery of co-primary neuropsychological tests as compared to patients randomized to placebo (multivariate analysis of combined cognitive outcome measures).

Furthermore, it is intended to investigate the hypothesis that Cerebrolysin, as compared to placebo, will show improved scores at four, seven and thirdteen months post-stroke on neurological deficit, global function, depression and anxiety. In addition, it is intended to investigate the effects of Cerebrolysin versus Placebo on the Quality of Live at seven and thirdteen months after stroke. Furthermore, it is intended to evaluate the safety of Cerebrolysin in patients with recent stroke and to determine which demographic, clinical and radiological characteristics predict response to treatment with Cerebrolysin.

Other study designs, including non-randomised controlled trials, can detect associations between an intervention and an outcome. But they cannot rule out the possibility that the association was caused by a third factor linked to both intervention and outcome. Random allocation ensures no systematic differences between intervention groups in factors, known and unknown, that may affect outcome. Double blinding ensures that the preconceived views of subjects and clinicians cannot systematically bias the assessment of outcomes. Intention to treat analysis maintains the advantages of random allocation, which may be lost if subjects are excluded from analysis through, for example, withdrawal or failure to comply. Meta-analysis of controlled trials shows that failure to conceal random allocation and the absence of double blinding yield exaggerated estimates of treatment effects.

4.1. Primary Objective

It is the primary objective of this clinical study to assess the efficacy of Cerebrolysin versus Placebo upon a battery of co-primary neurocognitive outcome scores at 180 and 360 days after baseline (7 and 13 months after the onset of ischeamic stroke).

4.1.1. Primary Variables

The following psychometric tests are combined by multivariate analysis with their score changes from baseline at 180 and 360 days after baseline (7 and 13 months after the onset of ischeamic stroke):

- Stroop Color Word test (Stroop)
- Trail-Making-Test Part A (TMT-A)
- Digit Span Backwards Task (DS-BW)
- Digit Symbol (DS-WPSI)

- Verbal Fluency Test – CFL Version (VFT-CFL)
- Rey Auditory Verbal Learning Test (RAVLT)

4.2. Secondary Objectives

It is the secondary objective of this clinical study to assess the efficacy of Cerebrolysin versus Placebo upon neurological deficit, functional outcome, symptoms of anxiety and depression, drug safety and quality of life at 180 and 360 days after baseline (7 and 13 months after the onset of ischeamic stroke).

4.2.1. Secondary Variables

- Score and score changes from baseline of the individual primary outcome scales at 180 and 360 days after baseline (7 and 13 months after the onset of ischeamic stroke)
- Score and score changes from baseline NIH Stroke Scale at 180 and 360 days after baseline
- Score and score changes from baseline Modified Rankin Score at 180 and 360 days after baseline
- Score and score changes from baseline Hospital Anxiety and Depression Scale at 180 and 360 days after baseline
- Score and score changes from baseline EQ-5D-5L at 180 and 360 days after baseline
- Safety parameters including Adverse Events at 180 and 360 days after baseline

5. STUDY DESIGN

Randomized, double-blind study design

One dose group (Cerebrolysin 30ml) shall be tested against placebo. The two study groups are therefore:

Study Group 1:	30ml Cerebrolysin
Study Group 2:	Placebo (0.9% NaCl)

The trial will be conducted in subjects suffering from ischemic stroke in the anterior circulation, either TACS or PACS. The diagnosis of the ischemic stroke as well as the location of the stroke will be determined clinically and confirmed by neuroimaging.

The study extends over an observation period of 390 days. Five visits for clinical evaluation are planned for the following points in time:

Visit 1 (Study Day -30±3 working days) – Screening Part 1 is scheduled within 72h after the onset of stroke and is followed by a 30 ± 3 working days run-in period for the patients. **Visit 2 - Screening Part 2 & Baseline Evaluation** is scheduled at study day 1. Following to the baseline assessment, study participants shall begin with Treatment Course 1 comprising ten once daily infusions of study medication on study days 1 to 10. Following a treatment free period of one month, patients shall receive Treatment Course 2 comprising ten once daily infusions of study medication on study days 61-70 and again after a break of one months Treatment Course 3 on study days 121-130. **Visit 3 – Efficacy & Safety Evaluation** which is scheduled for study day 180. The final **Visit 4 – Efficacy & Safety Evaluation** which is the primary endpoint of the study is scheduled for study day 360 post stroke. All treatment cycles and efficacy evaluations will be performed within a window of ±3 working days.

The study shall be initiated in May 2020. The first patient at each participating center should be enrolled within 2 months of initiation. The duration of the study is expected to be 4 years.

Clinical Study Phase: Phase IV

6. SELECTION AND WITHDRAWAL OF PATIENTS

6.1. Patient Inclusion Criteria – PART 1 / DAY -30

- Onset of Stroke within 72 hours prior to screening
- NIH Stroke Scale score between 5-15 at inpatient admission
- Pre-stroke mRS of 0 or 1
- No cognitive impairment prior to stroke with an IQ code score ≤ 3
- Age between 40 and 80 years, inclusive
- Patient is willing and able to comply with the protocol for the duration of the study

6.2. Patient Exclusion Criteria – PART 1 / Day -30

- Previous symptomatic ischaemic stroke or intracranial hemorrhage not related to the index stroke
- Severe visual or hearing impairment interfering with psychometric test procedures
- Pre-existing and active major neurological disease (eg. Parkinson's Disease, Epilepsy)
- Pre-existing and active major psychiatric disease, such as major depression, schizophrenia, bipolar disease, or dementia
- History of significant alcohol or drug abuse
- Advanced liver, kidney, cardiac, or pulmonary disease
- A terminal medical diagnosis with survival < 1 year
- Pregnancy or lactating
- Any contraindications to Cerebrolysin
- Current enrolment in another therapeutic study

6.3. Run-In Period – Day -30 to Day 1

During the Run-in-Period, the patient the patient shall received standard stroke care and the treatment administered will be recorded. Patients shall not receive Cerebrolysin treatment or treatment neuroprotective drugs during this period. Furthermore, an MRI will be performed before the study baseline visit.

6.4. Patient Inclusion Criteria – PART 2 / DAY 1

- Signed Informed Consent
- Patient continues to fulfill in- and exclusion criteria PART 1
- Diagnosis of stroke, ischemic in origin (TACS or PACS), confirmed by MRI

6.5. Patient Exclusion Criteria – PART 2 / Day 1

- Dementia due to strategic index stroke
- Major communication deficits with a Goodglass & Kaplan Score lower than 2
- Aphasia with an NIHSS Item 9 score of ≥ 2
- Treatment with Cerebrolysin or Neuroprotectants in the last 30 days
- Severe dementia with MMSE Score <12

6.6. Stopping and Discontinuation Criteria

6.6.1. Discontinuation Criteria related to the Study

- Insufficient recruitment
- Continuous serious protocol violation and deviation

6.6.2. Discontinuation Criteria related to the Patient

Patients will be advised in the Informed Consent Forms that they have the right to withdraw from the study at any time without prejudice and may be withdrawn at the Investigator's / Coordinator's discretion at any time. In the event that a patient drops out of the study or is withdrawn, the withdrawal / study termination page in the CRF should be completed. On the withdrawal page the Investigator should record the date of the withdrawal, the person who initiated withdrawal and the reason for withdrawal. Reasonable effort should be made to contact any patient lost to follow up during the course of the study in order to complete assessments and retrieve any outstanding data and study supplies.

Withdrawn by the Investigator due to

- Serious Adverse Drug Reaction
- Lack of efficacy
- Consent withdrawn
- Administrative reasons

The patient or his/her representative requested withdrawal due to

- An Adverse Event for which the Investigator did not consider removal from the study
- Perceived insufficient therapeutic effect.
- Withdrawal of consent for any other reason (data recorded until withdrawal will be kept in the database if not explicitly denied by the patient)

6.7. Randomisation, Blinding and Unblinding

This study will be performed under double-blind conditions to keep investigators, other study personnel and patients blinded to treatment allocation. Cerebrolysin is an amber-colored solution. Therefore, colored infusion lines will be used for drug administration.

Patients meeting in- and exclusion criteria will obtain a random number corresponding to the random list generated in advance by a biometrician selected by the coordinator. Patients will be randomly allocated to the study groups in a 1:1 ratio.

6.7.1. Production and Maintainance of Randomization Codes

Each presenting patient who qualifies for entry into the active treatment period is assigned a unique randomization number (patient number). This number is the next available randomization number in ascending order from 001 to e.g., 999 of a predefined randomization plan and identifies the treatment assigned to a unique patient in a double-blind way.

Patients are allocated to one of both treatment groups in a 1:1 ratio.

A balanced random code list is prepared using the random permuted block scheme. In accordance with the ICH Biostatistics Guideline, the block size is intentionally not given in the study protocol (ICH E9 § 2.3.2, “Investigators and other relevant staff should generally be blind to the block length”).

The sealed random code list and the sets of sealed envelopes are prepared using the validated program RANCODE in a validated working environment at idv Data Analysis and Study Planning, Gauting, Germany.

Sealed emergency envelopes will be provided to the Study Safety Officer (SSO) as well as to the Principle Investigator and the Study Nurse responsible for the preparation of the sudy medcation at each study site.

6.7.2. Blinded Preparation of Study Medication

The person who prepares the infusion at the study center will be independent of all other study specific procedures, in particular any safety or efficacy assessments and the study nurse is not allowed to disclose any information about treatment allocation.

The randomization envelope will be opened by the nurse at the time when the patient’s first ready-to-use-infusion is being prepared. The double-blind study medication labels of the ready-to-use-infusion will identify only the unique randomization number which is the same as the patient number.

6.7.3. Breaking the Randomization Codes / Unblinding

The Principle Investigator will receive a sealed envelope for each patient containing information as to the identity of the treatment dispensed. The randomization code for a patient may only be broken by the principal investigator for the following reasons:

- In the event of an SAE that the investigator feels cannot be treated without knowing the identity of the study medication
- If other reasonable suspicion of harm to the patient exist that requires knowledge of the study treatment

Every effort must be made to inform the designated Study Safety Officer prior to breaking the blind, or if it is an emergency, as soon as possible thereafter. Should unblinding be necessary, the randomization/emergency envelopes are dated (date, hour) and signed by the person who has opened the envelope and the investigator must provide a written explanation on the patient's CRF.

The whole study will be unblinded after closure of the database and finalization of the statistical analysis plan.

7. INVESTIGATIONAL PRODUCT

The Investigational Products will be made available by the study coordinator (FSNN).

7.1. Name and Description of the Investigational Product

Cerebrolysin Solution for Injection – 10 mL Ampoules
Active Ingredient: Cerebrolysin Concentrate

7.1.1. Dosage, Formulations and Administration

Cerebrolysin Solution for Injection

30 mL Cerebrolysin (contents of 3 x 10 mL Ampoules) is diluted in 0.9% saline solution up to total volume of 250 ml

Placebo

250 ml of 0.9% saline solution

The study medication will be infused over a period of 30 to 45 minutes. The study drug will be administered once daily by IV infusion for ten consecutive days starting on the day of the baseline examination. The first administration of study medication will be administered after all baseline assessment have been completed.

This treatment will be repeated with ten daily IV infusions on study days 1-10, 61-70, 121-130, and 241-250. Infusions will be given at approximately the same time on each day.

If a patient misses an infusion, it may be added at the end of each treatment course.

7.2. Packaging and Labelling

7.2.1. Study Material and Packaging

The Coordinator will provide the following study materials for each patient to the investigational center:

- Cerebrolysin 10ml ampoules
- 0.9% NaCl bags/vials
- IV lines (amber colored)
- Colored plastic sleeves

Cerebrolysin is provided in the form commercial drug product (10ml ampoules). It is packed in cardboard boxes containing 5 ampoules each. All other study materials will be supplied in bulk and will be used by the individual who prepares the study medication (ready-to-use infusion) as required. Therefore, no special packaging is needed for the study supplies.

7.2.2. Ready-to-use Study Medication

After preparation of the study medication, the Study Nurse will indicate the patient number as well as the date and time of the preparation of the study medication on the pre-printed label with a permanent marker pen.

This label will be put on the container of the infusion solution respectively on the bag used for blinding the study medication.

7.3. Storage

CRB should be kept and stored under 25 degrees Celsius, in its original package.

All supplies will be kept in a locked place, inaccessible to unauthorised persons until they are delivered to the individual patient.

7.4. Investigational Product Accountability and Destruction

The amount of used medication will be recorded in the CRF. All unused medication will be counted, recorded and destroyed upon completion of accountability.

7.5. Treatment Compliance

Since the infusion will be administered by a nurse or physician, documentation of the infusion procedure in the rating book will serve as the basis for compliance assessment.

8. CONCOMITANT THERAPY

All prior and concomitant medications must be recorded in the CRF including the date of onset, the stop date if applicable, the highest total daily dose and the route of administration. Furthermore, the reason (diagnosis) that is the basis for the intake of a specific concomitant treatment must be documented. Finally, if a concomitant medication is used as a prophylactic treatment, this must be indicated on the respective CRF page.

8.1. Allowed Concomittant Medication

The following concomitant treatments can be given if clinically necessary as per the investigator's judgement:

- Basic stroke treatment for general management of the patient, including thrombolysis, will be given on an as-needed basis, without restriction. The study will record concomitant medications (including dosage and frequency) at each visit.
- Compensation of fluid and electrolyte balance and acid-base balance
- Substances needed for adequate management of secondary symptoms including but not limited to antihypertensive agents, cardiovascular treatment, antidiabetic agents if necessary, treatment for sleep disturbances, antibiotics and body temperature lowering agents

8.2. Excluded Concomittant Medication

The following medications are not allowed during the study and every effort should be taken to avoid intake of substances listed below:

- Concomitant treatment with other neuroprotective or nootropic drugs (e.g. citicoline, memantine, amantadine, erythropoiethin, diazepam, investigational neuroprotective substances; piracetam, pramiracetam, pyritinol, meclosulfonat) except with those acting only peripherically.
- Concomitant treatment with substances that have a dilatory effect on blood vessels like naftidrofuryl, cinnarizine, flunarizine, nimodipine, nicergolin, pentoxifyllin, dihidroergotoxina (codergocrin), cinnarizine, naftidrofuryl), vinpocetin, vincamin or gingko biloba)
- Treatment with Cerebrolysin during the run-in-period of the study

8.3. Prior Medication

Chronically used prior medications should be kept at a constant dose throughout the duration of the trial if appropriate. Any changes of the prior medication must be documented in the CRF. Any neuroprotective drug treatment should be stopped before administration of study drug except for neuroprotective drugs acting only peripherically.

9. DEFINITION OF THE PRIMARY AND SECONDARY VARIABLES

9.1. Primary Variables

9.1.1. Stroop Color-Word Test

The Stroop Color-Word Test is based on the observation that individuals can read words much faster than they can identify and name colors. The cognitive dimension tapped by the Stroop is associated with cognitive flexibility, resistance to interference from outside stimuli, creativity, and psychopathology – all of which influence the individual's ability to cope with cognitive stress and process complex input. Whether the test is used as a screener or as part of a general battery, its quick and easy administration, validity, and reliability make it an especially attractive instrument. Furthermore, it is not culturally biased (Cohen, 2002). Thus, this unique test is an ideal way to screen for neuropsychological deficits. (Scarpina & Tagini, 2017)

9.1.2. Digit Span –Backward
Wechsler Adult Intelligence Scale, 4th Edition

The Digit Span task exercises a patient's verbal working memory. Attention and comprehension also contribute to performance. The digit span task is a common component of many IQ tests, including the widely used WAIS (Wechsler Adult Intelligence Scales). Performance on the digit span task is also closely linked to language learning abilities. The procedures for this assessment of working memory are considered standard. A list of numbers is read out loud at a rate of one number per second, and the participant is then asked to recall the numbers in order. The first list consists of three numbers and increases until the person begins to make errors. Lists with recognizable patterns (e.g., 1, 3, 5, 7, and 9) should be avoided, as people may remember these numbers more easily. At the end of each sequence, the participant is asked to the recall items in order. The average adult can remember a sequence of seven numbers, plus or minus two. This test can be distributed both backwards and forwards. Scores are thought to correlate with age and not intelligence. (Wechsler, 2008a)

9.1.3. Trial Making Test – B

The Trail Making Test is a neuropsychological test of visual attention and task switching. It consists of two parts in which the subject is instructed to connect a set of 25 dots (numbers/letters) in ascending order as quickly as possible while still maintaining accuracy. The test can provide information about visual search speed, scanning, speed of processing, mental flexibility, as well as executive functioning. The verison A consits of numbers while

the version B of the Trail-Making-Test consists of numbers and letters. (Soukup, Ingram, Grady, & Schiess, 1998)

9.1.4. Digit Symbol – Processing Speed Index, Wechsler Adult Intelligence Scale, 4th Edition

The proposed Digit Symbol test is a subpart of the Wechsler Processing Speed Index (PSI Digit Symbol Coding Subtest and PSI Symbol Search Subtest). Processing speed refers to the speed of cognitive processes and response output. The Processing Speed Index (PSI) is one of four indices that make up the full scale intelligence quotient (FSIQ) derived from The Wechsler Adult Intelligence Scale-4th edition (WAIS-IV) and The Wechsler Intelligence Scale for Children-4th edition (WISC-IV), primary standardized clinical instruments used to measure intelligence. The tasks included in the scales that comprise the PSI, (Coding, Symbol Search), are timed and require attending to visual material, visual perception and organization, visual scanning, and hand-eye coordination.

9.1.5. Verbal Fluency Test - Controlled Oral Word Association (COWA) Test - CFL Version

An oral Verbal Fluency Test was first developed by Arthur Benton over 40 years ago (Mitrushina, Boone, & D'Elia, 1998). It was included in the Multilingual Aphasia Examination (Benton & Hamsher, 1976) in a slightly different form and with a new name, the Con- trolled Oral Word Association (COWA) Test. This test, also known as the phonemic or letter fluency test, requires test takers to name as many words beginning with a single letter as they can in one minute. Standard administration provides three letters. This study will use the C-F-L version, as it provides less variability than F-A-S.

9.1.6. Rey Additory Verbal Learning Test

The Rey Auditory Verbal Learning Test (RAVLT) evaluates a wide diversity of functions: short-term auditory-verbal memory, rate of learning, learning strategies, retroactive, and proactive interference, presence of confabulation of confusion in memory processes, retention of information, and differences between learning and retrieval. Participants are given a list of 15 unrelated words repeated over five different trials and are asked to repeat. Another list of 15 unrelated words are given and the client must again repeat the original list of 15 words and then again after 30 minutes. Approximately 10 to 15 minutes is required for the procedure (not including 30 min. interval). (Schmidt, 1996)

9.2. Secondary Variables

9.2.1. Montreal Cognitive Assessment (MOCA)

Montreal Cognitive Assessment (MoCA) was designed as a rapid screening instrument for mild cognitive dysfunction. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills,

conceptual thinking, calculations, and orientation. Time to administer the MoCA is approximately 10 minutes. The total possible score is 30 points; a score of 26 or above is considered normal. (Nasreddine et al., 2005)

9.2.2. NIH Stroke Scale

The NIH Stroke Scale assesses neurologic deficit and is a 15 items scale that covers the level of consciousness, gaze, visual fields, facial palsy, motor functions, limb ataxia, aphasia, dysarthria and extinction and inattention (Brott et al, 1989). The NIHSS is observer-rated and takes 5-8 min to complete. Items have 3- to 5-point response scales, scored from 0 to 4 with higher score indicative of more severe disability. In case of patient death, the worst score possible will assigned. The NIHSS will be used to assess the severity of the stroke at baseline as well as in the follow-up examinations as a measure of neurological function deficit. (NIH, 2011)

9.2.3. Modified Rankin Score

The Modified Rankin Scale (Van Swieten et al, 1988) is a functional outcome scale measuring global outcome. It is used for grading the outcome and the level of disability after a stroke. The Modified Rankin Scale is a 7-point ordinal scale with a score of 0 indicative of no residual symptoms at all and the worst possible score of 6 which is assigned in case of death. The Modified Rankin Scale is observer rated and takes about 5 min to complete.

9.2.4. The Hospital Anxiety and Depression Scale (HADS)

A self-assessment scale has been developed and found to be a reliable instrument for detecting states of depression and anxiety in the setting of an hospital medical outpatient clinic. The anxiety and depressive subscales are also valid measures of severity of the emotional disorder. It is suggested that the introduction of the scales into general hospital practice would facilitate the large task of detection and management of emotional disorder in patients under investigation and treatment in medical and surgical departments. (Zigmond & Snaith, 1983)

9.2.5. EQ-5D-5L

The 5-level EQ-5D version (EQ-5D-5L) was introduced by the EuroQol Group in 2009 to improve the instrument's sensitivity and to reduce ceiling effects, as compared to the EQ-5D-3L. The EQ-5D-5L essentially consists of 2 pages: the EQ-5D descriptive system and the EQ visual analogue scale (EQ VAS). The descriptive system comprises five dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems and extreme problems. The patient is asked to indicate his/her health state by ticking the box next to the most appropriate statement in each of the five dimensions. This decision results in a 1-digit number that expresses the level selected for that dimension. The digits for the five dimensions can be combined into a 5-digit number that describes the patient's health state. (Herdman et al., 2011)

9.3. Screening Variables

9.3.1. Goodglass and Kaplan Communication Scale

This 6-point ordinal scale requires simple categorical assignment to determine the severity of an aphasia, which is based entirely on communicative ability. Categories and points are as follows:

0 No comprehensible speech expression and no comprehension of speech
1 Communication in fragmentary expression only: listener must dig for, ask about, or guess at the meaning of what is said. The amount of information that can be conveyed is limited and the interlocutor bears the main communicative burden.
2 A conversation on a familiar topic is possible with the help of the interlocutor. It is frequently impossible to express a thought. Patient and interlocutor still contribute approximately equally to the conversational content.
3 Patient can converse about nearly any everyday problem, requiring little or no support, although speech and comprehension disabilities disturb conversations on certain subjects or may even render them impossible.
4 Fluidity of speech is clearly reduced or speck comprehension is clearly limited. There is, however, no substantial disability affecting the form of content of speech.
5 Speech difficulties are hardly noticed. The patient may experience subjective difficulties of which the interlocutor is unaware. (Poeck, 1994)

9.3.2. IQ - CODE

Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) is a tool used to assess cognitive impairment in older people. The tool requires an informant to rate cognitive change over time on a 5 point likert scale. The IQCODE takes approximately 10-15 minutes to administer and is filled out by an informant. It can be used for people with lower levels of education and for those who are illiterate. (Ding et al., 2018)

9.3.3. MMSE

The Mini–Mental State Examination (MMSE) is a 30-point questionnaire that is used extensively in clinical and research settings to measure cognitive impairment. It is commonly used in medicine and allied health to screen for dementia. It is also used to estimate the severity and progression of cognitive impairment and to follow the course of cognitive changes in an individual over time (Folstein, 1975).

9.3.4. MRI

MRI will be performed using 1.5 or 3.0 Tesla equipment according to standard protocols and will be used to confirm the diagnosis of stroke ischaemic in origin as well as to document the location of the stroke and whether it is a small or large vessel stroke.

9.4. Safety Variables

9.5. Source Documents

Variable	Source document
Informed consent form (s)	Patient File
Patient's demographic data such as sex, age, weight, indication, concomitant diseases, medication history etc.	Patient File
Medical History	Patient File
Outcomes Variables	Patient File
CT	Patient File
MRI	Patient File
Concomittant Medication	Patient File
Adverse Events	Patient File

10. ASSESSING AND REPORTING OF ADVERSE EVENTS

Throughout the course of the clinical study particular attention is paid to the Adverse Events and Adverse Drug Reactions mentioned below.

10.1. Adverse Events (AE)

A Serious/Adverse Event (S/AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an Investigational Product, whether or not related.

10.2. Adverse Drug Reaction (ADR)

All untoward and unintended responses to an Investigational Product related to any application / dose administered. The phrase “responses to an Investigational Product” means having a reasonable causal relationship as judged by either the Investigator or the Coordinator. The expression reasonable means to convey in general that there is evidence or argument to suggest a causal relationship.

Regarding marketed Investigational Products: a response to a product which is noxious and unintended and which occurs at applications normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function.

10.2.1. Serious Adverse Event or Serious Adverse Reaction (SAE/SAR)

Serious Adverse Events will due to the underlying constitution of the patient be considered for AE documentation. Serious Adverse Drug Reactions will be dealt with as described below. Expedited Reporting is required if the following criteria apply (ICH E2A):

- Serious
- Unexpected
- Reasonable causal relationship to study treatment

An Adverse Drug Reaction is considered serious if it:

- Results in Death
- Is life threatening
- Requires additional inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability / incapacity
- Results in a congenital anomaly or birth defect

- Other medically significant event that requires immediate medical or surgical intervention

Unexpected is defined as:

- Not consistent with Investigators Brochure or SmPC

Causal Relationship is defined as:

- There are facts/evidence to suggest a causal relationship
- As judged by the reporting health care professional to have reasonable suspected causal relationship

Medical judgement should be exercised in deciding whether an Adverse Event / Reaction is serious in other situations. Important Adverse Events / Reactions that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Death: is the outcome of an Adverse Event. The event to be reported comprehensively is the medical condition leading to death, e.g. underlying disease, accident.

Life-threatening: in the definition of a Serious Adverse Event or Adverse Reaction refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.

10.3. Suspected Expected Serious Adverse Reaction (SESAR)

Any adverse reaction that is classed in nature as serious and which is consistent with the available information on the medicinal product in question set out in the SmPC

10.4. Suspected Unexpected Serious Adverse Reaction (SUSAR)

Any adverse reaction that is classed in nature as serious and which is not consistent with the available information on the medicinal product in question set out in the SmPC.

10.5. Recording of Adverse Events

All adverse events occurring after the start of the study must be reported, according to previously provided definitions, whether they are considered serious or not and regardless of the relationship to teh strudy medication will be documented in the CRF and were applicable reported. Subject entry into the study is defined as the time the informed consent is signed.

The nature of each individual AE, date and time of onset, duration, severity, relationship, any actions taken as well as outcome must be documented by the investigator. Additionally, it will be recorded whether the AE was serious nor not. Details of change to the dosing schedule or any corrective treatment must be recorded on the appropriate pages of the CRF.

The investigator is obliged to follow-up patients with AEs until the event has subsided, the condition is considered medically stable, or the patient is no longer available to follow-up. Patients who discontinue the study drug due to adverse experiences (clinical or laboratory) will be treated and followed according to established acceptable medical practice - all pertinent information concerning the outcome of such treatment will be entered on the CRF. AEs already documented in the CRF at a previous visit and designated as continuing must be reviewed at each visit. If an AE is resolved, documentation in the CRF must be completed to that effect. If an AE changes in frequency or severity during a study period, a NEW record of that experience will be initiated.

10.5.1. Definition of Adverse Event intensity

Intensity	Definition
Mild	Patient is aware of signs and symptoms, but they are easily tolerated
Moderate	Signs / symptoms cause sufficient discomfort to interfere with usual activities
Severe	Patient is incapable to work or perform usual activities

10.5.2. Definition of Adverse Event causality

On the basis of the WHO-UMC system for standardised case causality assessment (www.who-umc.org), the following categories are used to describe the degree of causality (all points should be complied with):

Definite

- Event or laboratory test abnormality, with plausible time relationship to drug intake
- Cannot be explained by disease or other drugs
- Response to withdrawal plausible (pharmacologically, pathologically)
- Event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognised pharmacological phenomenon)
- Re-challenge satisfactory, if necessary

Probable

- Event or laboratory test abnormality, with reasonable time relationship to drug intake

- Unlikely to be attributed to disease or other drugs
- Response to withdrawal clinically reasonable (for details refer to WHO-UMC)
- Re-challenge not required

Possible

- Event or laboratory test abnormality, with reasonable time relationship to drug intake
- Could also be explained by disease or other drugs
- Information on drug withdrawal may be lacking or unclear

Unlikely

- Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible)
- Disease or other drugs provide plausible explanations

Not related

- The event does not follow a reasonable temporal sequence from administration of the IMP and is clearly related to other factors, such as clinical state, therapeutic intervention or concomitant therapy

Not assessable

- Report suggesting an adverse reaction
- Cannot be judged because information is insufficient or contradictory
- Data cannot be supplemented or verified

All cases judged by any or both assessors as having a “reasonable causal relationship” to the IMP qualify as ADR. This corresponds to the categories “definite”, “probable” and “possible”.

10.6. Reporting of Serious Adverse Events

All Serious Adverse Reactions and all Unexpected Serious/Adverse Reactions with at least a suspicion of causal relationship to the investigational product must be reported to the Coordinator within 24 hours (one working day) of the Investigator becoming first knowledge. Preference in the reporting is the SAE report by e-mail to research@ssnn.ro.

10.7. Adverse Event/Reaction follow-up procedures

Adverse Events/ Reactions will be followed up throughout the course of the clinical study and any changes will be recorded in the CRF.

11. STUDY SCHEDULE

11.1. Procedures at Each Visit

Visit 1 – Screening PART 1 - within 72h after onset of the stroke (Study Day -30)

Visit 1 will be perfomed 30 ± 3 working days prior to randomization and the baseline assessment

- In/exclusion criteria – PART 1
  - CT
  - NIH Stroke Scale score between 5-15 at inpatient admission
  - IQ Code
- Demographic data
- Medical history
- Concomittant Medication

Run-In Period – Study Day -30 to Study Day 1

The Run-In period shall continue for 25 to 30 days beginning visit one. During the Run-In period the following will be performed

- MRI will be performed before study baseline
- Monitoring of concomitant medication
- Standard stroke treatment

Visit 2 – Screening PART 2 & Baseline Assessment – Study Day 1

- Informed Consent (signed up to Visit 2 start)
- In/exclusion criteria – PART 2
  - Mini-Mental State Examination (MMSE)
  - Goodglass & Kaplan Score
- Randomization
- Primary Evaluation Scales – Baseline Assessment
  - Stroop Color Word test (Stroop)
  - Trail-Making-Test Part A (TMT-A)
  - Digit Span Backward Task (DS-BW)
  - Digit Symbol (DS-WPSI)
  - Verbal Fluency Test – CFL Version (VFT-CFL)
  - Rey Auditory Verbal Learning Test (RAVLT)
- Secondary Evaluation Scales – Baseline Assessment
  - Montreal Cognitive Assessment (MOCA)

- Modified Rankin Scale
- NIH Stroke Score
- Hospital Anxiety and Depression Scale
- EQ-5D-5L

- Drug Safety Parameters
  - Adverse Events
- Concomittant Medication Monitoring

Treatment Cycle 1 – Study Day 1 to 10

During the first treatment cycle each patient shall receive 10 infusions, once daily on 10 consecutive days. The first infusion will be given after all baseline assessments have been performed. The following procedures will be performed during treatment visits:

- Adverse Event Monitoring
- Concomittant Medication Monitoring

Treatment Cycle 2 – Study Day 61 to 70

During the second treatment cycle each patient shall receive 10 infusions, once daily on 10 consecutive days. The following procedures will be performed during treatment visits:

- Adverse Event Monitoring
- Concomittant Medication Monitoring

Treatment Cycle 3 – Study Day 121 to 130

During the second treatment cycle each patient shall receive 10 infusions, once daily on 10 consecutive days. The last infusion will be given on the day of Visit 3 before the assessments The following procedures will be performed during treatment visits:

- Adverse Event Monitoring
- Concomittant Medication Monitoring

Visit 3 – Efficacy & Safety Assessment – Study Day 180

- Primary Evaluation Scales – Baseline Assessment
  - Stroop Color Word test (Stroop)
  - Trail-Making-Test Part A (TMT-A)
  - Digit Span Backward Task (DS-BW)
  - Digit Symbol (DS-WPSI)
  - Verbal Fluency Test – CFL Version (VFT-CFL)
  - Rey Auditory Verbal Learning Test (RAVLT)

- Secondary Evaluation Scales – Baseline Assessment
  - Montreal Cognitive Assessment (MOCA)
  - Modified Rankin Scale
  - NIH Stroke Score
  - Hospital Anxiety and Depression Scale
  - EQ-5D-5L
- Drug Safety Parameters
  - Adverse Events
- Concomittant Medication Monitoring

Treatment Cycle 4 – Study Day 241 to 250

During the second treatment cycle each patient shall receive 10 infusions, once daily on 10 consecutive days. The following procedures will be performed during treatment visits:

- Adverse Event Monitoring
- Concomittant Medication Monitoring

Visit 4 – Efficacy & Safety Assessment – Study Day 360

- Primary Evaluation Scales – Baseline Assessment
  - Stroop Color Word test (Stroop)
  - Trail-Making-Test Part A (TMT-A)
  - Digit Span Backward Task (DS-BW)
  - Digit Symbol (DS-WPSI)
  - Verbal Fluency Test – CFL Version (VFT-CFL)
  - Rey Auditory Verbal Learning Test (RAVLT)
- Secondary Evaluation Scales – Baseline Assessment
  - Montreal Cognitive Assessment (MOCA)
  - Modified Rankin Scale
  - NIH Stroke Score
  - Hospital Anxiety and Depression Scale
  - EQ-5D-5L
- Drug Safety Parameters
  - Adverse Events
- Concomittant Medication Monitoring

11.2. Assessment of Compliance

Compliance will be documented by recording the date and time of the administration in the CRF. The number of IV infusions actually administered to each patient will be calculated as the percentage of the total number of IV infusions planned per protocol and will provide a measure of treatment compliance. All treatment cycles and efficacy

evaluations will be performed within a window of ±3 working days.

11.3. Risk assessment and Precautionary Measures

The investigational medicinal product is clinical use for many years and has demonstrated a very benign safety profile. The safety information for the IMP is provided in the SmPC in Appendix 1.

12. STUDY AND TREATMENT DURATION

Study/Treatment start: 05 / 2020
Study/Treatment end: 12 / 2025

13. STATISTICAL METHODS

The final statistical analysis of the study will be performed by a qualified biometrician and will fulfill all ICH/GCP requirements for handling of clinical study data. The statistical analysis, including any subgroup analysis will be agreed upon prior to data evaluation and the results will be fixed in a statistical analysis plan (SAP). The study data will be analysed and the Statistical Report written as soon as all study data are entered into the study data base and the entered data are validated.

13.1. Preliminary Remark

Although this study is intended to be of exploratory nature, the analysis will be based on 'confirmatory' principles with pre-specification of the primary analyses and control of multiple level alpha.

13.2. Primary Objective

It is the primary objective of this study to assess the efficacy of Cerebrolysin versus Placebo upon a battery of co-primary neurocognitive outcome scores at 6 and 12 months after baseline.

13.3. Primary Efficacy Criteria

13.3.1. Justification for Multi-Dimensional Approach

To address the multidimensional breadth of cognitive impairment/recovery in patients after stroke, the primary endpoint of trial is defined as a multidimensional cognitive endpoint, combining the various cognitive dimensions by a global, multidimensional, correlation-sensitive approach. This way the primary cognitive objective can be defined in terms of a combination of individual effects across the single cognitive endpoints, thus substantially enhancing the assay sensitivity and strength of evidence. The procedure is the generalized Wilcoxon-Mann-Whitney test (Wei-Lachin procedure).

13.3.2. Defined Efficacy Ensemble

The following ensemble of appropriate single efficacy criteria shall be tested by a multivariate, directional test approach, reflecting the cognitive status of patients with Acute Ischemic Stroke at 180 and 360 days after baseline:

Multivariate Efficacy Ensemble

- Stroop test (Stroop)
- Trail-Making-Test Part A (TMT-A)

- Digit Span Backwards Task (DS-BW)
- Digit Symbol (DS-WPSI)
- Verbal Fluency Test – CFL Version (VFT-CFL)
- Rey Auditory Verbal Learning Test (RAVLT)

13.4. Secondary Objectives

It is the secondary objective of this clinical study to assess the efficacy of Cerebrolysin versus Placebo upon a battery of the co-primary neurocognitive outcome scores at 180 and 360 days after baseline as well as to assess the efficacy of Cerebrolysin versus Placebo on neurological deficit, functional outcome, symptoms of anxiety and depression and drug safety at 180 and 360 days after baseline.

13.5. Secondary Variables

- Score and score changes from baseline of the individual primary outcome scales at 180 and 360 days after baseline
- Score change from baseline of the NIH Stroke Scale at 180 and 360 days after baseline
- Modified Rankin Score at 180 and 360 days after baseline
- Score change from baseline of the Hospital Anxiety and Depression Scale at 180 and 360 days after baseline
- EQ-5D-5L at 180 and 360 days after baseline.
- Drug Safety parameters including Adverse Events

13.6. Level of Significance

The multiple level alpha is set to $\alpha = 0.05$, two-sided.

13.7. Multiplicity

Multiplicity regarding multiple primary outcome measures is controlled by the chosen correlation-sensitive, multivariate test procedure (Wei-Lachin procedure, see also section 13.9).

Mutiplicity regarding the two primary points in time is controlled by means of the principle of *a priori* ordered hypotheses (fixed sequence approach). The procedure of *a priori* ordered hypothesis is most powerful with full control of alpha (for control of alpha using stepwise testing see Maurer W, Hothorn LA, Lehmacher W 1995). The sequence and nature of the a priori ordered test-statistical hypotheses of the trial is defined as follows:

1. Combined cognitive outcome measures as defined in section 13.3.2, score changes from baseline to day 180 (month 4 post stroke), multivariate analysis, generalized Wilcoxon-Mann-Whitney test (Wei-Lachin procedure)
2. Combined cognitive outcome measures as defined in section 13.3.2, score changes from baseline to day 360 (month 13 post stroke), multivariate analysis, generalized Wilcoxon-Mann-Whitney test (Wei-Lachin procedure)

The principle of *a priori* ordered hypotheses is embedded in the well-known general principle of closed testing. The pre-planned chain of hypotheses can be tested in a confirmatory way with the same full alpha value; each test result smaller than alpha will be statistically significant as long as the preceding test result was also significant.

13.8. Sample Size Calculation

The power for this study is determined based on the following design specifications:

- Two-sided type I error defined as alpha = 0.05, two-sided (multiple level alpha, see section 13.6)
- Type II error defined as β = 0.1 (Testpower 90%)
- Design alternative effect size: Mann-Whitney statistic (MW) = 0.60 (small to medium-sized difference according to Cohen (Colditz, 1988); assuming a normal distribution the effect size MW may easily be re-expressed as the well-known Cohen effect size (Cohen, 1988) of a standardized difference (Cohen's d): MW = 0.60 means Cohen's d = 0.35)
- Estimated correlations among the single outcome scales included in the global statistics ρ = 0.6)

Nonparametric sample size calculations within the framework of a multiple outcome approach (Wei-Lachin procedure- Wei and Lachin, 1984; Lachin 1992) was performed applying the validated software Nnpar 1.0 from idv Data Analysis and Study Planning, Krailling/Munich (see also Tang, 1999; Lachin, 1981). A good example for sample size calculation according to the Lachin approach (Wei and Lachin, 1984; Lachin 1992; Lachin, 1981) in multidimensional trials with neuroprotective agents is also given by Huang (2008).

Please note: further details of the effect size (Mann-Whitney statistic) are described in section 13.9, further details of sample size assessment are described in the separate document "Sample Size Assessment Based on a Multidimensional Efficacy Approach".

Based on the above design specifications, the total required sample size for the multivariate ensemble results in 145 patients per group (including 15% enhancement for usual „ambiguities“, e.g., dropouts). With this sample size a „small to medium-sized“ group difference (MW = 0.60) with regard to the multivariate cognitive outcome ensemble can be detected with a power of 90%.

13.9. Confirmatory Analyses

Minimizing the required assumptions is a recommended approach for confirmatory statements on efficacy (LaVange, 2005). This applies especially in scales with skewed distributions including floor and ceiling effects as is known from many cognitive scales. Furthermore, data types can be of different nature (binary, ordinal, continuous). Thus, a nonparametric assessment of treatment effects independent of data type and distribution is the method of choice for the primary analysis.

The nonparametric analysis will be performed using the Wei-Lachin procedure, a multivariate generalization of the Wilcoxon-Mann-Whitney test, which takes account of the correlation among univariate Mann-Whitney tests for each outcome to produce an overall average estimate of benefit and test for treatment differences. The summarizing test used is, however, not the undirectional or omnibus test of the classical procedure, but instead the directional test which is most efficient in the case of known direction for superiority (as is the case for the selected outcome scales).

The procedure is described by Wei and Lachin (1984), Lachin (1992), and Lachin (2014). Practical examples are given in modern textbooks on multiple testing problems (see e.g., Dimitrenko, 2009). Incidentally it should be noted that the nonparametric Wei-Lachin procedure is similar to the frequently used parametric procedure of O’Brien (O’Brien, 1984). We prefer, however, the Wei-Lachin procedure as it is more robust for practical data sets (minimization of required assumptions (LaVange 2005)) and because the O’Brien procedure has been shown to give too liberal results (Frick 1997).

It is important to note, that the multivariate, directional test procedure chosen for this study can cope simultaneously with binary, ordinal and continuous data. Thus, there is no technical need for the widely used dichotomization of original scales which is associated with substantial loss of information and reflects a major disadvantage of previous Stroke studies.

The effect size measure directly associated to the Wilcoxon-Mann-Whitney test is the Mann-Whitney statistic. It is recommended by many authors for its sensitivity and robustness in all data situations (Agresti et al. 1984, Brunner et al. 2000, Munzel et al. 2003, D`Agostino et al. 2006, Brunner et al. 2013, Kieser et al. 2013). For

ordinal/rating scales it is regarded as method of choice or 'gold standard', see also Design and Analysis of Non-Inferiority Trials (Rothmanns et al. 2011).

Technically, the MW gives the probability that a randomly chosen patient of the test group is better off than a randomly chosen patient of the comparison group (with the probability ranging from 0 to 1, with 0.5 indicating equality); it is statistically defined as: P (X<Y) + 0.5 P (X=Y).

Applying the Mann-Whitney effects size measure, the null and alternative hypothesis for the comparisons of the test treatment to control treatment (superiority) can be formulated as follows:

H_0: MW_{TC} 0.50

H_A: MW_{TC} 0.50

H_0: Null-hypothesis; H_A: Alternative Hypothesis; T: Test Treatment; C: Control

Treatment; MW: Mann-Whitney Effects Size Measure

The traditional benchmarks for the Mann-Whitney effects size measure (MW) are as follows (Colditz,1988):

0.29	large inferiority
0.36	medium inferiority
0.44	small inferiority
0.50	equality
0.56	small superiority
0.64	medium superiority
0.71	large superiority

The confirmatory analyses are performed with the FAS population according to the ICH Guideline E9 (full analysis set). Since the use of the per protocol set, however, maximises the opportunity for a new treatment concept to show additional efficacy in the analysis, and most closely reflects the scientific model underlying the protocol (see ICH E9, section 5.2.2), the supportive analysis by means of the per-protocol set will be regarded as of equal scientific importance (see also Schwartz 1967, Schwartz 1980, Senn 2007).

13.10. Exploratory Analyses

All primary and secondary efficacy criteria will be analyzed with descriptive group statistics.

In addition, nonparametric effect sizes and confidence intervals (Mann-Whitney effects size measure) will be provided for all primary and secondary efficacy criteria at all points in time.

13.11. Accounting for missing data

13.11.1. Missing Data Problems – General Considerations

Missing data are a problem in every data analysis. Of course, there are always missing data of the type ´missing completely at random´ (MCAR), which in principle will not bias the results; the analysis procedure should be able to cope with partially missing data of such a type. In many studies this type of data is treated by LOCF replacement (Last Observation Carried Forward) as far as there exist follow-up measurements at previous visits.

In a study like the one planned there might also be informatively missing data (missing not at random, MNAR): participants of the study died or are unable to complete the tests because of brain-related impairment. Neglecting these missing data might introduce bias.

A reasonable policy for minimizing bias in the case of informatively missing data (MNAR) is the replacement of these missing data by assigning the worst possible score, or a score worse than those observed. It should be noted that this strategy is only reasonable if rank-based robust procedures are used for the data analysis.

The worst rank imputation procedure was recommended by Lachin in his seminal paper about the missing data problem for data missing because of mortality when performing an exercise test (Lachin, 1999). This procedure was also used when analyzing non-fatal outcomes in studies where mortality was a problem (Lusben, 2002; McMahon, 2001). Recently a similar procedure has been proposed by the ´Traumatic Brain Injury (TBI) Clinical Trials Network´ when designing the COBRIT study (Bagiella, 2010).

Temkin (2007) included deaths with the worst rank for the significance tests of neuropsychological scales but excluded deaths for the calculation of descriptive statistics (e.g., mean, SE estimates). Thus, significance tests reflect all patients with estimation of missing data while the descriptive statistics reflect only the actually observed assessments. We prefer not excluding deaths from descriptive statistics, since the study treatment with more deaths would artificially have better neuropsychological scores while a study treatment preventing deaths would be burdened by rather severe scores of survived patients. The use of robust descriptive statistics in this study allows the inclusion of worst rank scores for deaths also in

descriptive analysis. This way, confirmatory analyses and descriptive analysis can be based on the same analysis data and contradictory results are avoided.

13.11.2. Handling of Missing Data

In order to identify each type of missing data, outcome scales will be coded for every patient and visit according to the following scheme (see also Bagiella, 2010):

1 = valid (complete task)
2 = unable to complete (stroke-related neurological reason) [describe reason]
3 = not completed (different reasons, not stroke related) [describe reason]

13.11.3. Worst Rank Imputation

For outcome scales with code “2” a worst rank imputation will be introduced for the corresponding patients since these data are informatively missing (missing not at random, MNAR). These missing data are replaced by the worst possible score of the corresponding outcome scale.

13.11.4. LPCF Imputation

For outcome scales with code “3” a LPCF replacement will be introduced (Last Percentile Carried Forward) as far as previous follow-up evaluations exist. This method carries forward the actual status information of the patient population, using the percentile value with back transformation to raw scale, instead of last value carried forward. This approach was recently developed and recommended by O´Brien, Zhang and Bailey (2005) for the analysis of data from chronic, progressive diseases as dementia. According to their simulation study the calculated estimators should be negligibly biased by missing data. If no general change of patients over time occurs the method is more or less identical with LOCF (Last Value Carried Forward), if change occurs bias is minimized.

If no previous follow-up measurement exists, the outcome scale remains missing. It is important to note that the chosen multivariate test procedure (Wei-Lachin procedure) can handle partially missing single scales of type MCAR (missing completely at random).

13.12. Definition of study population

13.12.1. General Issues

Before the study is unblinded, a blind review will be performed. In this process, possible protocol violations will be classified as “severe”, “major”, “minor“, or “none”. Patients will be allocated to the individual data sets with regard to the classification of

possible protocol violations. The analysis populations (Safety, ITT, and PP) will be listed individually in the final statistical analysis plan.

13.12.2. Safety Population

Safety population includes all patients who have had at least one dose of study medication and one contact with the Investigator afterwards. It will be used for safety analysis.

13.12.3. Full Analysis Set (FAS)

FAS population is defined as all patients who have no “severe” violation of entry criteria, had at least one dose of medication and at least one post-baseline observation of at least one primary efficacy criterion (definition according to ICH E9 § 5.2. Analysis Sets). FAS population will be used for all efficacy analyses.

13.12.4. Per Protocol Population (PP)

A sensitivity analysis will be performed for a per protocol (PP) data set as an exploratory approach. The PP population includes all patients who are eligible for FAS evaluation and who additionally do not show major protocol deviations. As noted in section 13.9, the supportive analysis by means of the per-protocol set will be regarded as of equal scientific importance as the FAS analysis, since it most closely reflects the scientific model underlying the protocol (see ICH E9, section 5.2.2).

13.13. Homogeneity Analyses (Exploratory Interpretation)

Homogeneity analyses for baseline shall be performed based on the FAS population.

In addition to descriptive analyses robust nonparametric Mann-Whitney effects size measures and their two-sided 95% confidence intervals shall present an overview on demographic-anamnestic variables and on the primary efficacy criteria at baseline. This allows comparison of baseline variables across different scales and data types.

As benchmark for relevant baseline differences, a Mann-Whitney effects size measure of 0.36 and 0.64 respectively will be applied (referring to a standardized difference of 0.5 according to Cohen, which is regarded as a medium-sized difference).

In the case of heterogeneities, stratified analyses will be performed as second line analyses.

13.14. Compliance

Patients with compliance for the entire study below 80% for the treatments will be considered protocol violators and will not be included in the per protocol analysis.

13.15. Blind Review and Final Statistical Analysis Plan

A blind review of the data shall be performed within the framework of the requirements of the ICH Guideline E9. The statistical analysis plan will be finalized by the statistician before the decoding takes place. The analysis populations (Safety, FAS, and PP) will be listed individually in the final statistical analysis plan.

Formal records will be kept of when the statistical analysis plan was finalised as well as when the blind was subsequently broken.

13.16. Software Applied

Nonparametric sample size calculation was performed applying the validated software Nnpar 1.0 from idv Data Analysis and Study Planning, Gauting/Munich.

The data analysis will be performed in a validated working environment according to the requirements of the ICH-Guidelines E3 (1995). The software to be used for data evaluation will be described in the final statistical analysis plan.

14. ACCESS TO SOURCE DATA / DOCUMENTS

The Investigator will permit study-related monitoring, audits, IRB / IEC review and regulatory inspections, providing direct access to primary patient data (i.e. source data) which supports the data on the CRFs for the study, i.e. general practice charts, appointment books, original laboratory records etc.

14.1. Source Data

Source data are defined as all information in original records and certified copies of original records of clinical findings, observations or other activities in a clinical study necessary for the reconstruction and evaluation of the study. Source data are contained in source documents (original records or certified copies).

14.2. Source Documents

Source documents are defined as original documents, data and records (e.g. hospital records, clinical and office charts, laboratory notes, memoranda, patient diaries or evaluation check lists, pharmacy dispensing records, recorded data from automated instruments, copies or manuscripts certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, patient files, records kept at pharmacy, at the laboratories and at medico technical departments involved in clinical study).

14.3. Direct Access

Direct access is defined as the permission to examine, analyse, verify and reproduce any records and reports that are important to evaluation of a clinical study. Any party (e.g. domestic and foreign regulatory authorities, the Coordinator and / or authorised representatives of the Coordinator such as monitors and auditors) with direct access should take all reasonable precautions within the constraints of the applicable regulatory requirements to maintain the confidentiality of patient identities and Coordinator proprietary information.

15. QUALITY CONTROL AND QUALITY ASSURANCE

15.1. Quality Control

Quality Control is defined as the operational techniques and activities, such as monitoring, undertaken within the quality assurance system to verify that the requirements for quality of the study related activities have been fulfilled.

Quality Control should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

15.2. Study Monitoring

Authorized, qualified Clinical Trial Monitor will visit the investigational site in regular intervals, established based on the needs of the project, to verify adherence to protocol and local legal requirements, to perform source data verification and to assist the Investigator in his study related activities.

15.3. Quality Assurance

Quality Assurance is defined as the planned and systematic actions that are established to ensure that the study is performed and the data are generated, documented (recorded) and reported in compliance with Good Clinical Practice (GCP) and the applicable regulatory requirements.

15.4. Inspection

An Inspection is defined as the act by an authority (IRB/IEC) of conducting an official review of documents, facilities, records and any other resources that are deemed by the authorities to be related to the clinical study and that may be located at the site of the study, or at the Coordinators and / or clinical research organisation facilities or at any other establishments deemed appropriate by the authorities.

15.5. Audit

An audit is a systematic and independent review of study related activities and documents to determine whether the validated study related activities were conducted and the data were recorded, analysed and accurately reported according to the protocol, designated Standard Operating Procedure (SOPs), Good Clinical Practice (GCP) and the applicable regulatory requirements. An independent audit at the study site may take place at any time during or after the study.

15.6. 13.5 Risk-Based Centralized Statistical Monitoring

In accordance to the most recent requirements of the ICH E6 Guideline for Good Clinical Practice (GCP, Amendment R2, July 2015), the EMA reflection-paper on Risk-based Quality Management in Clinical Trials (2013), and the FDA Guidance for Industry on a Risk-based Approach to Monitoring (2013), a risk-based centralized statistical approach to monitoring is introduced in combination with targeted on-site monitoring for ongoing surveillance of study conduct, thus ensuring highest standards of data quality and integrity.

16. ETHICAL AND LEGAL CONSIDERATIONS

16.1. Ethical Considerations

Before initiating a study, the Investigator will have written and dated approval / favourable opinion from the relevant IRB / IEC for the study protocol as well as for any amendments. Approval will be indicated in writing with reference to the final protocol number and date. Details of the IRB / IEC's constitution including names of its members and their function in the committee (e.g. chairman, specialist, lay-member) should be made available for inclusion in the Trial Master File.During the study all documents that are subject to review should be provided to the IRB / IEC by the Investigator.

16.2. Independent Ethics Committee (IEC) / Institutional Review Board (IRB)

The study protocol including all amendments and the study CRF will be submitted to the IRB/EC of the study centre before initiation of the study. IRB/EC approval for the study protocol and all amendments will be obtained prior to the start of any study specific procedures.

16.3. Informed Consent

Patients will be informed about the study procedures and potential risks and benefits of the study. Their consent to participate in this study will be obtained before any study-specific procedures are carried out. A sample informed consent is provided in Appendix 2.

16.4. Modification of Protocol

The Investigator or the Coordinator should not implement any deviation from, or changes of, the protocol without mutual agreement, prior review and documented approval from the IEC of a respective amendment. The only exceptions are where necessary to eliminate an immediate hazard to study patients, or when the changes involve only logistical or administrative aspects of the study (e.g. change in monitor(s), change of telephone number(s)).

The party initiating an amendment must confirm it clearly in writing and it must be signed and dated by the Coordinator and the Coordinating Investigator. Necessary protocol amendments will be submitted to the appropriate IECs and can only be implemented after a favourable opinion from the IEC has been obtained and is documented in writing.

16.5. Conduct of Study

This clinical study will be conducted in accordance with the Declaration of Helsinki. It will be conducted in compliance with this protocol, Good Clinical Practice (2001/20/ EEC,

CPMP/ICH/135/95), designated Standard Operating Procedures, and with local laws and regulations relevant to the use of investigational new drugs in the country of conduct.

16.6. Personal Data and Data Protection

All data obtained in the context of the clinical study are subject to data protection. The patient's name in addition to other data related to persons (excluding date of birth / age and sex) are not to be disclosed by the Investigator or the investigating physicians. The latter shall take care that the case report forms or other documents (e.g. copies of reports on special findings) transmitted to the FSNN contain no names, but another identifier. The storage of data for statistical assessment shall be performed under the patient's identifier. Only the Investigator and the investigating physicians can perform assignment of the identifier to the personal data.

If it becomes necessary in the course of the study to identify a patient's name for medical reasons, all the individuals involved are subject to an obligation to maintain secrecy.

If personal data are stored and processed, the requirements of data protection legislation are to be observed.

16.7. Data Handling and Record Keeping

16.7.1. Completion of Case Report Forms

Any data to be recorded directly into the CRFs will be identified at the start of the study.

The investigator must ensure the accuracy, completeness legibility and timeliness of data reported in the CRF and all required reports. Any change or correction to a paper CRF must be dated, initialled and explained (in case of an eCRF data entries are already monitored by an audit trail) and must not obscure the original entry, this applies to both written and electronic changes.

Data reported on the CRF that are derived from source documents must be consistent with the source documents or the discrepancies should be explained.

Within two weeks after completion of each patient, the Investigator should agree to have completed and signed CRFs available for full inspection by the clinical monitor.

16.7.2. Archiving

On termination of the study, the study documents, including the emergency envelopes are to be returned to the Coordinator. These records are to be retained for the periods required by ICH-GCP, i. e. until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the Investigational Product (CPMP/ICH/135/95), or by national legal

requirements, whichever is longer, but not less than 15 years after routine/premature termination of a clinical study.

The final report shall be retained for at least 2 years after the Investigational Products are removed from the last market. The informed consent forms and all the original (raw) data are to be retained by the head of the clinical study or the investigating physicians for at least 15 years.

16.8. Confidentiality

The aim and contents of the study, in addition to its results are to be treated as confidential by all persons involved in the clinical study.

16.9. Responsibilities

The responsibilities of the Investigator, Monitor and Coordinator of the clinical study as regards handling of data, storage of data, planning, assessment and quality assurance are regulated by the recommendations on ”Good Clinical Practice” of the ”International Conference on Harmonisation” (ICH) and apply to this clinical study.

17. FINAL REPORT AND PUBLICATION POLICY

It is intended that the results of the study may be published as scientific literature. Results may also be used in submissions to regulatory authorities. The following conditions are to protect commercial confidential materials (patents, etc.), not to restrict publication.

All information concerning the Investigational Product (such as patent applications, formulae, manufacturing processes, basic scientific data, or formulation information supplied to the Investigator by the Coordinator and not previously published) is considered confidential and shall remain the sole property of the Coordinator. The Investigator agrees not to use it for other purposes without the Coordinator's written consent.

It is understood by the Investigator that the Coordinator will use the information developed in this clinical study in connection with the development of the Investigational Product and therefore may be disclosed as required to other Investigators or any appropriate international Regulatory Authorities. In order to allow for the use of information derived from this clinical study, the Investigator understands that he/she has an obligation to provide the Coordinator with complete test results and all data developed during this study.

18. REFERENCES

Adams, H. P., Bendixen, B. H., Kappelle, L. J., Biller, J., Love, B. B., Gordon, D. L., & Marsh, E. E. (1993). Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke, 24(1), 35–41.

Agresti, A. 1984. Analysis of Ordinal Categorical Data, New York, NY, Wiley & Sons.

Bagiella E. et al. Clinical Trials in Rehabilitation: Single or Multiple Outcomes? Arch Phys Med Rehabil 2009; 90(11 Suppl 1):17-21

Bagiella E, Novack TA, Ansel B, Diaz-Arrastia R, Dikmen S, Hart T, Temkin N. Measuring outcome in TBI treatment trials: Recommendations from the TBI Clinical Trials Network. J Head Trauma Rehabil 2010

Brunner, E., Munzel, U., The nonparametric Behrens-Fisher problem: Asymptotic theory and a small sample approximation, Biom. J. 42 (2000) 17-25

Brunner, U., Munzel, E. Nichtparametrische Datenanalyse, Springer, Berlin 2013

Cohen, J., Statistical power analysis for the behavioural sciences, Lawrence Erlbaum Associates, Hillsdale, New York 1988

Colditz, G.A, Miller, J.N., Mosteller, F., Measuring gain in the evaluation of medical technology: The probability of a better outcome, In. J. Technol. Assessment Health Care 4 (1988) 637-642

D'Agostino, Campbell M, Greenhous, J. (ed.). The Mann-Whitney statistic: continuous use and discovery; Special Papers for the 25th Anniversary of Statistics in Medicine. Statistics in Medicine 2006; Volume 25.

Damulin, I. V., Koberskaya, N. N., & Mkhitaryan, E. A. (2008). Effects of cerebrolysin on moderate cognitive impairments in cerebral vascular insufficiency (a clinical-electrophysiological study). Neuroscience and Behavioral Physiology, 38(6), 639–645. https://doi.org/10.1007/s11055-008-9014-5

Ding, Y., Niu, J., Zhang, Y., Liu, W., Zhou, Y., Wei, C., & Liu, Y. (2018). Informant questionnaire on cognitive decline in the elderly (IQCODE) for assessing the severity of dementia in patients with Alzheimer's disease. BMC Geriatrics, 18. https://doi.org/10.1186/s12877-018-0837-9

Dmitrienko, A., Tamhane, A.C., Bretz, F., Multiple testing problems in pharmaceutical statistics, CRC Press, New York, 2009

European Medicine Agency. Reflection Paper on risk based quality management in clinical trials, EMA/269011/2013,

Fagerland, M.W., Sandvik, L., The Wilcoxon-Mann-Whitney test under scrutiny, Statist. Med. 28 (2009) 1487-1497

FDA. Guidance for Industry: Oversight of Clinical Investigations - A Risk-Based Approach to Monitoring, U.S. Department of Health and Human Services, Food and Drug Administration. August 2013
Frick H. A note on the bias on O'Brien's OLS test. Biometrical J. 1997; 39:125-128.

Folstein, MF; Folstein, SE; McHugh, PR (1975). ""Mini-mental status". A practical method for grading the cognitive state of patients for the clinician". Journal of Psychiatric Research. 12 (3): 189–98. doi:10.1016/0022-3956(75)90026-6. PMID 1202204.

Frick, H., A maxmin linear test of normal means and its application to Lachin's data. Communications in Statistics -Theory and Methods 23 (1994) 1021-1029

Frick, H., Nonparametric comparisons of medians of survival functions, Statistical Software Newsletter in CSDA 27, 1998, 357-361

Frick, H., Undominated p-values and property C for unconditional one-sided two sample binomial tests. Biom. J. 42, (2000), 715-728

Herdman, M., Gudex, C., Lloyd, A., Janssen, M., Kind, P., Parkin, D., … Badia, X. (2011). Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Quality of Life Research: An International Journal of Quality of Life Aspects of Treatment, Care and Rehabilitation, 20(10), 1727–1736. https://doi.org/10.1007/s11136-011-9903-x

Huang, P., Woolson, R. F., O´Brien P. C., A rank-based sample size method for multiple outcomes in clinical trials, Statist. Med. 27 (2008) 3084-3104

ICH Harmonised Guideline. Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice - E6(R2). 11 June 2015.

ICH-Biostatistics Guideline, ICH Topic E9, ICH Harmonized Tripartite Guidance, Note for Guidance on Statistical Principles for Clinical Trials. September 1998; CPMP/ICH/363/96.

Kahler, E., Rogausch, A., Brunner, E., Himmel, W., A parametric analysis of ordinal quality-of-life data can lead to erroneous results, J. Clinic. Epidem. 61 (2008) 475-480

Kieser M. Statistical methods for the assessment of clinical relevance. In K. van Montfort, J. Oud and W. Ghidey (Eds.): Developments in Statistical Evaluation of Clinical Trials. Chapter 11, p. 195-207, Springer, 2014

Kieser, M., Friede, T., Gondan, M., Assessment of statistical significance and clinical relevance, Statist. Med. 32 (2013) 1707-1719

Lachin J.M., Some large-sample distribution-free estimators and tests for multivariate partially incomplete data from two populations. Stat. Med. 11 (1992) 1151-1170

Lachin JM. Biostatistical Methods. The Assessment of Relative Risks, Wiley, New York 2000; Chapter 9.

Lachin JM. Introduction to sample size determination and power analysis for clinical trials. Controlled Clinical Trials. 1981; 2:93-113.

Lachin JM. Worst-rank score analysis with informatively missing observations in clinical trials. Controlled Clinical Trials. 1999;20:408-422.

Lachin, J.M., Applications of the Wei-Lachin multivariate one-sided test for multiple outcomes on possibly different scales. PLoS ONE 9 (2014); doi:10.1371/journal.pone.0108784

Larry Rubinstein, John Crowley, Percy Ivy, Michael LeBlanc, Dan Sargent. Randomized Phase II Designs. Clin Cancer Res. 2009 March 15; 15(6): 1883–1890. doi:10.1158/1078-0432.CCR-08-2031.

Levine, D. A., Galecki, A. T., Langa, K. M., Unverzagt, F. W., Kabeto, M. U., Giordani, B., & Wadley, V. G. (2015). Trajectory of Cognitive Decline After Incident Stroke. JAMA, 314(1), 41–51. https://doi.org/10.1001/jama.2015.6968
Mann, H. B., Whitney, D. R., On a test of whether one of two random variables is stochastically larger than the other, Ann. Math. Statistic.18 (1947) 50–60

Matsouaka, R.A., Betensky, R.A., Power and sample size calculations for the Wilcoxon-Mann-Whitney test in the presence of death-censored observations, Statist. Med. 34 (2015) 406-431

Matula, C., & Schoeggl, A. (2000). Cerebral protection before, during and after neurosurgical procedures. Stereotactic and Functional Neurosurgery, 75(2–3), 142–146. https://doi.org/10.1159/000048396

Maurer, W., Hothorn, L.A., Lehmacher, W., Multiple comparisons in drug clinical trials and precli-nical assays: a-priori ordered hypotheses, in: Testing Principles in Clinical and Pre-clinical Trials, Hrsg. Von J. Vollmar, Biometrie in der chemisch-pharmazeutischen Industrie 6, Gustav Fischer, Stuttgart (1995) p. 3-18

Munzel, U., Bandelow, B., The use of parametric vs. nonparametric tests in the statistical evaluation of rating scales, Pharmacopsychiat. 31 (1998) 222-224

Munzel, U., Hauschke, D. A nonparametric test for proving noninferiority in clinical trials with ordered categorical data. Pharmaceutical Statistics, 2 (2003), 31-37.

Nasreddine, Z. S., Phillips, N. A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., … Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: A Brief Screening Tool For Mild Cognitive Impairment. Journal of the American Geriatrics Society, 53(4), 695–699. https://doi.org/10.1111/j.1532-5415.2005.53221.x

O'Brien, P.C., Procedures for comparing samples with multiple endpoints, Biometrics 40 (1984) 1079-1087

Pocock, S.J., Geller, N.L., Tsiatis, AA, The analysis of multiple endpoints in clinical trials, Biometries 43 (1987) 487-498

Polushin, A. Y., Yanishevskiy, S. N., Maslevtsov, D. V., Krivov, V. O., Beskrovnaya, O. V., & Molchan, N. S. (2017). [The efficacy of prevention of postoperative cognitive dysfunction in cardiac surgeries with the use of the cerebrolysin]. Zhurnal Nevrologii I Psikhiatrii Imeni S.S. Korsakova, 117(12), 37–45. https://doi.org/10.17116/jnevro201711712137-45

Rahlfs V.W., Zimmermann, H., Lees, K.L., Effect size measures and their relationships in stroke studies, Stroke. 2014; 45: 627-633

Rahlfs, V.W., Vester, J.C., Der multidimensional Ansatz statt Testung einzelner Endpunkte, Pharm. Med. 14 (2012) 160-165

Rosenberger, W.F., Lachin, J.M., Bain, R.P., Nonparametric test of stochastic ordering for multiple longitudinal measures, J. Biopharmac. Statist. 5 (1995) 235-243

Rothmann, M.D., Wiens, B.L., Chan, I.S.F., Design and Analysis of Non-Inferiority Trials, Boca Raton, CRC Press 2012

Scarpina, F., & Tagini, S. (2017). The Stroop Color and Word Test. Frontiers in Psychology, 8. https://doi.org/10.3389/fpsyg.2017.00557

Soukup, V. M., Ingram, F., Grady, J. J., & Schiess, M. C. (1998). Trail Making Test: issues in normative data selection. Applied Neuropsychology, 5(2), 65–73.
https

Sprent, P., Smeeton, N. C., Applied Nonparametric Statistical Methods, 3 edition, Chapman and Hall, Boca Raton 2001
://doi.org/10.1207/s15324826an0502_2

Stephens, T. L. (2014). The assessment of executive functioning using the Delis-Kaplan Executive Functions System (D-KEFS). In S. Goldstein & J. A. Naglieri (Eds.), Handbook of executive functioning (pp. 209-222). New York, NY, US: Springer Science + Business Media.

Tang, D.-I., Geller, N.L., Closed testing procedures for group sequential clinical trials with multiple endpoints, Biometrics 55 (1999) 1188-1192

Vereshchagin, N. V., Suslina, Z. A., Timerbaeva, S. L., Kashina, E. M., Gnezditskiĭ, V. V., Maksimova, M. I., … Smirnova, I. N. (2001). [Treatment and prevention of cognitive dysfunction in patients with arterial hypertension and atherosclerosis: results of a randomized double-blind placebo-controlled trial of cerebrolysin]. Terapevticheskii Arkhiv, 73(4), 22–27.

Wechsler, D. (2008a). Wechsler Adult Intelligence Scale—Fourth Edition. San Antonio, TX: Pearson Assessment.

Wei L.J., Lachin J.M., Two-sample asymptotically distribution-free tests for incomplete multivariate observations. J. Am. Stat. Assoc. 79 (1984) 653-661

Wilcoxon, F., Individual comparisons by ranking methods, Biometrics Bull.1 (1945) 80-83
Zimmermann, H., Rahlfs, V.W., Nonparametric methods in standard statistics packages: The case of the Friedman analysis, SSN (Statistical Software Newsletter) in CSDA 20 (1995) 97-106

Zigmond, A. S., & Snaith, R. P. (1983). The hospital anxiety and depression scale. Acta Psychiatrica Scandinavica, 67(6), 361–370.

- **SIGNATURES**

The undersigned have read this protocol and agreed to conduct this study in accordance with all stipulations of the protocol and in accordance with the Declaration of Helsinki.

Date: **Signature:**

_____ **Prof. Dr. Dafin F Muresanu**

(Coordinating Investigator)

_____ **Dr. Adina Dora Stan**

(Principal Investigator)

_____ **Dr. Olivia Verisezan-Rosu**

(Study Coordinator)