CLINICAL STUDY PROTOCOL

CEREBROLYSIN AND REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION (RTMS) IN PATIENTS WITH TRAUMATIC BRAIN INJURY

STUDY IDENTIFICATION:

Study Code:	CAPTAIN-rTMS
Protocol Number:	FSNN040418

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Romania

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

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1. ABBREVIATIONS AND DEFINITIONS

Please add study specific abbreviations and definitions.

e.g.:

AE	Adverse Event
A(D)R	Adverse (Drug) Reaction
C	*Control*
CANTAB	*Cambridge Neuropsychological Test Automated Battery*
CRB	Cerebrolysin
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Computed tomography
DLPFC	Dorso-lateral pre-frontal cortex
ET	Eye tracking
FSNN	Foundation for the Study of Neuroprotection and Neuroplasticity
GCP	Good Clinical Practice
GCS	Glasgow Coma Scale
H	Hour
HA	Alternative Hypothesis
HAM-A	Hamilton Anxiety Rating Scale
HAM-D	Hamilton Rating Scale for Depression
H0	Null-hypothesis
Hz	Hertz
ICH	International Conference for Harmonization
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
incl.	including
IRB	Institutional Review Board

IUD	Intrauterine device
ITT	Intention-to-treat
IV	Intra-venous
LOCF	Last Observation Carried Forward
LPFC	Last Percentile Carried Forward
MCAR	Missing completely at random
mL	Milli Liter
MoCA	Montreal Cognitive Assessment Scale
MNAR	Missing not at random
MRI	Magnetic resonance imaging
MTT	Multi-tasking test
MW	Mann-Whitney
NA	Not Applicable
OTS	One Touch Stocking of Cambridge
PLC	Placebo
PP	Per protocol
PSI	Processing Speed Index
qEEG	Qantitative electroencephalography
rTMS	Repetitive transcranial magnetic stimulation
RT	Reaction Time
SAP	Statistical analyses plan
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
T	Test Treatment

TBI	Traumatic Brain Injury
WAIS	Wechsler Adult Intelligence Scale
WHO-UMC	World Health Organization-Uppsala Monitoring Center

2. PROTOCOL SUMMARY / SYNOPSIS

Title	
Investigational Medicinal Product	*R-TMS + Cerebrolysin Solution for Injection*
Number of Sites & Countries	1 site, 1 country
Phase	II
Indication	Traumatic Brain Injury
Study Design	monocentric, randomized, double-blind, phase II study
Study Duration	Study start: 04/2018 Study end: 12/2020
Sample Size	• Treatment Group CRB + rTMS: N=30 • Treatment Group CRB + sham rTMS: N=30 • Treatment Group placebo + sham rTMS: N=30
Primary Objective	To *assess the efficacy of the combin*ed *rTMS and Cerebrolysin treatment versus CRB alone, upon* a battery of *neurocognitive outcomes* at 3 and 6 months post TBI
Secondary Objectives	To assess the single efficacy criteria at three and six months post TBI To test ET and qEEG parameters as biomarkers of cognitive dysfunction To assess the safety of rTMS administrated starting with one month after TBI

	To check assay sensitivity for the primary objective (rTMS + CRB versus CRB alone) by comparing CRB alone versus PLC.
Primary Variables	Processing Speed Index, Stroop Color-Word Test, Trail Making Test, Digit Span, Montreal Cognitive Assessment, One Touch Stockings of Cambridge (CANTAB), Reaction Time (CANTAB), Multitasking Test (CANTAB), Hamilton Rating Scale for Depression, Hamilton Anxiety Rating Scale)
Secondary Variables	Eye tracking parameters Quantitative EEG parameters rTMS adverse events
Inclusion Criteria	• Traumatic brain injury onset 30 days prior to screening • CT/MRI – focal and/or diffuse lesions • Age: 18-70 years, inclusive • Pre-Trauma Karnofsky Index 100 • Patient is willing and able to comply with the protocol requirements for the duration of the study
Exclusion Criteria	• Metal implant in the head or within the stimulation area • Medical implanted devices (cardiac pacemaker, cochlea implant or medication pumps) • History of intracranial interventions as well as ischemic or hemorrhagic stroke • Evidence of pre-existing major health problems (e.g., cancer, hematological, renal, hepatic, or coronary disease, psychiatric disorder, diabetes, myocardial infarction or other known heart diseases, disabling or musculoskeletal problems as rheumatoid arthritis,

	epilepsy, evidence of degenerative or inflammatory diseases affecting nervous system [e.g., Alzheimer, Parkinson]). Patients with well controlled diabetes and hypertension can be included if there is no evidence of secondary damage to major organs. • Any neurological or non-neurological condition independent from TBI that might influence the functional outcome or other efficacy outcome measures. • Injury of writing hand influencing cognitive or other outcome measures, in the investigator's judgment. • Clear clinical signs of intoxication influencing the evaluation, in the investigator's judgment. • Major drug dependency including alcohol, in the investigator's judgment. • Chronic treatment with steroids, Ca2+-channel blockers or major anticoagulants (e.g., warfarin and other coumarin derivates), monoamine oxidase inhibitors, antipsychotic drugs or nootropic molecules. • Patient with penetrating brain injury. • Females who are pregnant or lactating. • Females who are of child bearing potential and not taking adequate contraceptive precautions are excluded from the trial. Females of child bearing potential taking acceptable contraceptive precautions can be included. A highly effective method of birth control and one which is acceptable for this study, is defined as those which result in a low failure rate (i.e. less than 1% per year) when used consistently and correctly such as implants, injectables, combined oral contraceptives, some IUDs, sexual abstinence or vasectomised partner.
Visit Schedule	**Screening and Baseline** – Study day 0 (within 30 days after TBI onset)

	• Neurological and physical exam • Hematology and blood chemistry • Demographic data • Medical history • Evaluation Scales: Processing Speed Index, Stroop Color-Word Test, Trail Making Test, Digit Span, Montreal Cognitive Assessment, One Touch Stockings of Cambridge (CANTAB), Reaction Time (CANTAB), Multitasking Test (CANTAB), Hamilton Rating Scale for Depression, Hamilton Anxiety Rating Scale • ET • qEEG **Treatment cycles** • Study day 31-40 • Study day 61-70 • Study Day 91-100 **Visit 1** – Efficacy Evaluation (Study Day 101) • Neurological and general exam • Evaluation Scales: Processing Speed Index, Stroop Color-Word Test, Trail Making Test, Digit Span, Montreal Cognitive Assessment, One Touch Stockings of Cambridge (CANTAB), Reaction Time (CANTAB), Multitasking Test (CANTAB), Hamilton Rating Scale for Depression, Hamilton Anxiety Rating Scale • ET • qEEG **Visit 2** – Efficacy Evaluation (Study Day 180)

	• Neurological and general exam • Evaluation Scales: Processing Speed Index, Stroop Color-Word Test, Trail Making Test, Digit Span, Montreal Cognitive Assessment, One Touch Stockings of Cambridge (CANTAB), Reaction Time (CANTAB), Multitasking Test (CANTAB), Hamilton Rating Scale for Depression, Hamilton Anxiety Rating Scale • ET • qEEG
Investigational Product Characteristics	• IV Cerebrolysin (CRB), 30 ml diluted in 0.9% saline solution up to 250 ml, daily during 10 consecutive working days; three treatment cycles during study days 31-40, 61-70 and 91-100) • The rTMS treatment will be performed daily during 10 consecutive working days; three treatment cycles during study days 31-40, 61-70 and 91-100) The device MagPro X100 (MagVenture, Denmark) will be used for repetitive stimulation with a figure-8 coil (MCF-B65). The coil will be hold tangential to the scalp with the handle pointing upwards. For localizing the left dorsolateral prefrontal cortex (DLPFC) stimulation, we will use the 10-20 EEG system by placing the coil at F3. The stimulation parameters for DLPFC will be set up at 10 Hz and 1,200 stimuli/day, with an intensity of 120% of resting motor threshold. The resting motor threshold is determined at the beginning of the first treatment session and is defined as the minimal intensity at which at least five of 10 motor evoked potentials are 50 μV in amplitude in the pollicis abductor brevis.

Reference Product	Standard TBI treatment

3. INTRODUCTION

3.1. Background information

After an acute brain lesion, there is always an endogenous continuous brain defense response consisting of two main sequences: an immediate one that aims to reduce brain damage, known as neuroprotection, and a later one that aims to repair the brain damage, known as neurorecovery, which is based on neurotrophicity, neuroplasticity and neuro-genesis.

Neurotrophic factors are the most important endogenous molecules involved in brain protection and recovery. They are modulating molecules with immediate pleiotropic neuroprotective activity and long-term multimodal effects. Due to this unique therapeutic effect, the principle of treatment with neurotrophic factors is based on, in addition to acute administration, repetitive periods of treatment. This principle is applied to stimulate long-term endogenous capacity for neurorecovery that is induced by neurotrophicity, neuroplasticity and neurogenesis.

Cerebrolysin has a neurotrophic factor-like activity based on the four important endogenous neurobiological processes: neurotrophicity, neuroprotection, neuroplasticity and neurogenesis. Additionally, this activity may have similar effects as the real sequence of endogenous post-lesional regulation.

Transcranial magnetic stimulation (TMS) operates on Faraday's principle of electromagnetic induction.

There are several studies regarding a beneficial role of repetitive TMS (rTMS) in neurorehabilitation, including in TBI patients such as: motor recovery including spasticity, depression treatment, and speech rehabilitation (Fitzgerald, 2011; Bonni, 2013; Castel-Lacanal, 2013, Krewer, 2014).

According with the paradigm that rTMS effects are based on the induction of potential actions, most of the studies regarding the effect of rTMS on cognition are based on the idea that stimulating with different frequencies on a certain area, will activate/deactivate specific regions or even networks and so will enhance/inhibit specific functions. However, the experimental studies, showed that rTMS influence also the molecular and cellular level, influence that can be independent from the induction of action potentials. One of the key targets by which rTMS improves cognitive function appears to be BDNF, magnetic stimulation having a stimulating effect on its genetic expression.

Even that rTMS has extensively been tested in patients with Alzheimer's disease (Liao, 2015), in TBI there is a little date about rTMS in cognitive rehabilitation.

A recently published article described the effects of rTMS in various post-concussive symptoms, including cognitive dysfunction. There were administrated 20 sessions of RTMS (20 x 5-sec trains; 10-Hz at 110% threshold), with clinical and functional magnetic resonance imaging (fMRI) assessments before and after intervention, and clinical assessment at 3-month follow-up, in 15 patients with mild TBI. The results showed cognitive improvement and a decrease of post-concussive symptoms (Koski, 2014). The use of high frequency stimulation on DLPFC is concordant with data existing in literature in TBI, dementia, stroke and depression (Miniussi, 2011; Koski, 2014, Nadeau, 2014). There is a single case presented in literature with rTMS treatment for depression in TBI which represent a common complication in this pathology and is independently associated with decreased cognitive functions (Fitzgerald, 2011).

The main area of concern regarding the use of TMS in stroke or TBI patients has been the triggering of kindling activity, which can induce seizures Seizure induction, however, has rarely been reported following rTMS, and animal studies have shown that there is no clear evidence that rTMS leads to increased seizure susceptibility (Miniussi and Rossini, 2011).

3.2. Study Rationale

Cognitive treatment with rTMS was tested in Alzheimer's diseases and the combination between rTMS combined with cognitive treatment seems to have a beneficial effect upon cognition (Bentwich, 2011). Regarding rTMS as an add-on to pharmacological treatment in cognitive rehabilitation, there are too few data to establish its efficacy. (Haffen, 2012). There are several studies on rTMS as add-on treatment in depression, with good results when the magnetic stimulation was performed with high frequencies (Mogg, 2008; Chen, 2013). In TBI, this study is the first one in order to test the efficacy of the combining treatment rTMS + pharmacological intervention (CRB) in cognitive rehabilitation.

4. STUDY OBJECTIVES

This study shall assess the efficacy of the combining treatment rTMS + pharmacological intervention (CRB) in cognitive rehabilitation of patients with traumatic brain injuries.

4.1. Primary Objective

It is the primary objective of this clinical study to assess the efficacy of the combining rTMS and Cerebrolysin treatment versus CRB alone, upon a battery of co-primary neurocognitive outcomes at 3 and 6 months post TBI.

4.1.1. Primary Variables

The scores and/score changes from baselines at 3 and 6 months post TBI of the following clinical scales: Processing Speed Index, Stroop Color-Word Test, Trail Making Test, Digit Span, Montreal Cognitive Assessment, One Touch Stockings of Cambridge (CANTAB), Reaction Time (CANTAB), Multitasking Test (CANTAB), Hamilton Rating Scale for Depression, Hamilton Anxiety Rating Scale

4.2. Secondary Objectives

- To assess the single efficacy criteria at 3 and 6 months post TBI.
- To test ET and qEEG parameters as biomarkers of cognitive dysfunction.
- To assess the safety of rTMS administrated starting with one month after TBI.
- To check assay sensitivity for the primary objective (rTMS + CRB versus CRB alone) by comparing CRB alone versus PLC.

4.2.1. Secondary Variables

- Eye Tracking (ET) parameters
- Quantitative EEG (qEEG) parameters
- Safety Parameters (laboratory values, adverse events)

5. STUDY DESIGN

Monocentric, randomized, double-blind, phase II study

6. SELECTION AND WITHDRAWAL OF PATIENTS

6.1. Patient Inclusion Criteria

- Traumatic brain injury onset 30 days prior to screening
- CT/MRI– focal and/or diffuse lesions
- Age: 18-70 years, inclusive
- Pre-Trauma Karnofsky Index 100
- Patient is willing and able to comply with the protocol requirements for the duration of the study

6.2. Patient Exclusion Criteria

- Metal implant in the head or within the stimulation area
- Medical implanted devices (cardiac pacemaker, cochlea implant or medication pumps)
    - History of intracranial interventions as well as ischemic or hemorrhagic stroke
    - Evidence of pre-existing major health problems (e.g., cancer, hematological, renal, hepatic, or coronary disease, psychiatric disorder, diabetes, myocardial infarction or other known heart diseases, disabling or musculoskeletal problems as rheumatoid arthritis, epilepsy, evidence of degenerative or inflammatory diseases affecting nervous system [e.g., Alzheimer, Parkinson]). Patients with well controlled diabetes

and hypertension can be included if there is no evidence of secondary damage to major organs.

- Any neurological or non-neurological condition independent from TBI that might influence the functional outcome or other efficacy outcome measures.
- Injury of writing hand influencing cognitive or other outcome measures, in the investigator's judgment.
- Clear clinical signs of intoxication influencing the evaluation, in the investigator's judgment.

6.3. Stopping and Discontinuation Criteria

6.3.1. Discontinuation Criteria related to the Study

- *Insufficient recruitment*
- *Continuous serious protocol violation and deviation*

6.3.2. 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 / Sponsor'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).

6.4. 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.

A set of envelopes for each patient enrolled should be distributed to the study nurse preparing the ready-to-use-infusion solution. These nurses are only responsible for the preparation and administration of infusion solutions, and they should not be involved in any further study-related procedures. This person should not be allowed to disclose any information about treatment allocation. A treatment envelope should not be opened until the patient's first ready-to-use-infusion has been prepared.

Sham stimulation will be performed with a sham-coil (MCF-P-B 65, Magventure) which has a mechanical outline and sound level identical to MCF-B65, and also provides the same level of cutaneous discofort and muscle twiching as real stimulation. The rTMS (both sham and real) administration will be provided by two rTMS technicians which will not be involved in any further study-related procedures and will not be allowed to disclose any information about treatment procedure.

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 sponsor. Based on the random list sealed, opaque randomization/emergency envelops will be provided as follows:

- To the study centre to break blinding if reasonable suspicion of harm to the patients exists

- To the person assigned to prepare the read-to-use-infusion
- To the person assigned to administrate the rTMS protocol.
  -
  - To the study coordinator

On opening, the randomization/emergency envelopes are dated (date, hour) and signed by the person who has opened the envelope. The Investigator should promptly document and explain to the Sponsor any premature unblinding of the Investigational Product(s). The whole study will be unblinded after closure of the database and determination of the analysis populations.

7. INVESTIGATIONAL PRODUCTS

The Investigational Products will be made available by the sponsor (FSNN)

7.1. Name and Description of the Investigational Products

Cerebrolysin Solution for Injection

Repetitive Transcranial Magnetic Stimulation (rTMS)

7.1.1. Dosage, Formulations and administration

Cerebrolysin Solution for Injection

IV Cerebrolysin (CRB), 30 ml diluted in 0.9% saline solution up to 250 ml, daily during 10 consecutive working days with 3 treatment cycles during study days 32-40, 61-70 and 91-100).

Repetitive Transcranial Magnetic Stimulation (rTMS)

The rTMS treatment will be performed daily during 10 consecutive working days with 3 treatment cycles during study days 32-40, 61-70 and 91-100).

The medical device MagPro X100 (MagVenture, Denmark) will be used for repetitive stimulation with a figure-8 coil (MCF-B65). The coil will be hold tangential to the scalp with the handle pointing upwards. For localizing the left dorsolateral prefrontal cortex (DLPFC) stimulation, we will use the 10-20 EEG system by placing the coil at F3. The stimulation parameters for DLPFC will be set up at 10 Hz and 1,200 stimuli/day, with an intensity of 120% of resting motor threshold. The resting motor threshold is determined at the beginning of the first treatment session and is defined as the minimal intensity at which at least five of 10 motor evoked potentials are 50 μV in amplitude in the pollicis abductor brevis. For detailed information (SmPCs) on these products please refer to Appendix 1.

7.2. Packaging and Labelling

The investigational product and the reference product will be packaged and labelled for use in clinical trial according to GMP Annex 13 and local legislation.

7.3. Storage

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

All supplies must 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 and documented and unused investigational products will be destroyed upon completion of accountability.

8. CONCOMITANT THERAPY

Not allowed concomitant medication: monoamine oxidase inhibitors, antipsychotic drugs or nootropic molecules.
All concomittant medications and therapies will be recorded in the CRF.

9. DEFINITION OF THE PRIMARY AND SECONDARY VARIABLES

9.1. Primary Variables

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.

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.

Processing Speed Index, Wechsler Adult Intelligence Scale, Third Edition

The Processing Speed Index (PSI) assesses skills such as focusing attention and quick scanning as well as discriminating between and sequentially ordering visual information. It requires persistence and planning ability, but it is sensitive to motivation, difficulty working under time pressure, and motor coordination as well. It is also related to reading, mathematical, and memory

skills. Cultural factors seem to have little impact on processing speed. Processing Speed (PS) refers to the speed at which cognitive processes can be performed.

Digit Span, Wechsler Adult Intelligence Scale, Third 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.

Trial Making Test

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 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 Hamilton Anxiety Rating Scale

The Hamilton Anxiety Rating Scale (HAM-A) is a psychological questionnaire used by clinicians to rate the severity of a patient's anxiety. The scale consists of 14 items designed to assess the severity of a patient’s anxiety. Each of the 14 items contains a number of symptoms, and each group of symptoms is rated on a scale of zero to four, with four being the most severe. All of these scores are used to compute an overarching score that indicates a person’s anxiety severity.

The evaluator is instructed to assess the extent to which the patient displays the given criterion. Each item is scored independently based on a five-point, ratio scale. A rating of 0 indicates that the feeling is not present in the patient. A rating of 1 indicates mild prevalence of the feeling in the patient. A rating of 2 indicates moderate prevalence of the feeling in the patient. A rating of 3 indicates severe prevalence of the feeling in the patient. A rating of 4 indicates a very severe prevalence of the feeling

in the patient. To implement the Hamilton Anxiety Rating Scale, the acting clinician proceeds through the fourteen items, evaluating each criterion independently in form of the five-point scale described above. Upon the completion of the evaluation, the clinician compiles a total, composite score based upon the summation of each of the 14 individually rated items. This calculation will yield a comprehensive score in the range of 0 to 56. It has been predetermined that the results of the evaluation can be interpreted as follows. A score of 17 or less indicates mild anxiety severity. A score from 18 to 24 indicates mild to moderate anxiety severity. Lastly, a score of 25 to 30 indicates a moderate to severe anxiety severity.

Hamilton Rating Scale for Depression

Hamilton Rating Scale for Depression (HAM-D, is a multiple item questionnaire used to provide an indication of depression, and as a guide to evaluate recovery. The questionnaire is designed for adults and is used to rate the severity of their depression by probing mood, feelings of guilt, suicide ideation, insomnia, agitation or retardation, anxiety, weight loss, and somatic symptoms.

One Touch Stockings of Cambridge

One Touch Stockings of Cambridge is a test of executive function, based upon the Tower of Hanoi test. It assesses both the spatial planning and the working memory subdomains.

Administration time: 10 minutes. Task format: The participant is shown two displays containing three coloured balls. The displays are presented in such a way that they can be easily perceived as stacks of coloured balls held in stockings or socks suspended from a beam. This arrangement makes the 3-D concepts involved apparent to the participant and fits with the verbal instructions. There is a row of numbered boxes along the bottom of the screen. The test administrator first demonstrates to the participant how to move the balls in the lower display to copy the pattern in the upper display and completes one demonstration problem, where the solution requires one move. The participant must then complete three further problems, one each requiring two moves, three moves and four moves. Next the participant is shown further problems and must work out in their head how many moves the solutions require and then select the appropriate box at the bottom of the screen to indicate their response.

Multitasking Test

The Multitasking Test is a test of the participant’s ability to manage conflicting information provided by the direction of an arrow and its location on the screen and to ignore task-irrelevant information.

Administration time: 8 minutes

Task format: The test displays an arrow which can appear on either side of the screen (right or left) and can point in either direction (to the right or to the left).

Each trial displays a cue at the top of the screen that indicates to the participant whether they have to select the right or left button according to the “side on which the arrow appeared” or the “direction in which the arrow was pointing”. In some sections of the task this rule is consistent across trials (single task) while in others it may change from trial to trial in a randomised order (multitasking). Using both rules in a flexible manner places a higher demand on cognition than using a single rule. Some trials display congruent stimuli (e.g. arrow on the right side pointing to the right) whereas other trials display incongruent stimuli, which require a higher cognitive demand (e.g. arrow on the right side of the screen pointing to the left).

Reaction Time (RTI)

Reaction Time provides assessments of motor and mental response speeds, as well as measures of movement time, reaction time, response accuracy and impulsivity.

Administration time: 3 minutes. Task format: The participant must select and hold a button at the bottom of the screen. Circles are presented above (one for the simple mode, and five for the five-choice mode.) In each case, a yellow dot will appear in one of the circles, and the participant must react as soon as possible, releasing the button at the bottom of the screen, and selecting the circle in which the dot appeared.

9.2. Secondary Variables

Eye Tracking

Eye movements will be recorded by a human infrared eye tracking system (Tobii TX300) with 300 Hz temporal resolution. The target stimulus, which will be created in Toby Studio program, will be presented on a computer screen 40 cm from the subject, in a darkened room. Before testing, an eyechart will be used to verify that all subjects will have a normal or corrected-to-normal vision. Calibration based on 9 points, including center and peripheral, will be performed before each session, which also will ensured that all subjects will have a full range of oculomotor movement.

32 Channel – qEEG

Continuous qEEG recordings will be performed in both resting conditions and cognitive tasks, during the following sequences: 1) eyes open – 5 minutes; 2) eye close – 5 minutes; 3) cognitive tests – 20 minutes; 4) eyes open -5 minutes; 5) eyes closed - 5 minutes. Will be used scalp electrodes fixed in an elastic cap, located according to the international 10–10 system and electrode impedances will be keep below 5 kΩ. qEEG data will be recorded using 32 channels.

9.3. Further Variables

Neurological and physical examinations will be performed according to hospital standard procedures and will be recorded in the CRF.

Anamnestic data will be collected according to standard hospital procedures and the medical history will be documented in the CRF.

Vital signs including systolic and diastolic blood pressure, heart rate, respiratory rate and body temperature will be measured and documented in the CRF.

Standard laboratory parameters will be analysed according to standard procedures.

Information on all concomitant treatments and medications will be collected and documented.

Information on adverse events and patient safety will be collected in the CRF.

9.4. Source Documents

The following definitions of source documents shall apply:

Variable	Source document
Informed consent form (s)	Informed consent form

Variable	Source document
Patient's data (e.g. demographics: sex, age, weight, indication, concomitant diseases, medication history etc.)	CRF
Medical History	CRF
Physical & Neurological Examination	CRF
Vital signs	CRF
Outcomes Variables	CRF
Laboratory Variables	CRF
qEEG	Electronic Patient File
ET	Electronic Patient File
Concomittant Medication	CRF
Adverse Events	CRF

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 Sponsor. 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):

1. Serious
2. Unexpected
3. 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 means:

- Not consistent with Investigators Brochure or SmPC

Causal Relationship means:

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

NOTE

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.

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.

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, according to previously provided definitions, whether they are considered serious or not will be documented and were applicable reported.

The Investigator must report in detail all adverse signs and symptoms which are either volunteered by patients or observed during or following the course of Investigational Product administration on the appropriate CRF page.

Included in the description should be the nature of the sign or symptom; the date of onset; date of resolution (duration); the severity / intensity; the relationship to study treatment or other therapy; the action taken (if any), and the outcome.

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 reasonably 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 Sponsor within 24 hours (one working day) of the Investigator becoming first knowledge. Preference in the reporting is the SAE report by e-mail, however fax may be also used:

FSNN representative for pharmacovigilance reporting aspects:

Dr. Anca Moldovanu (contact details to be added)

10.7. Exemption from expedited reporting

not applicable

10.8. 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

Screening and Baseline Visit – within 30 days of onset of TBI (Study Day 0)

- Neurological and physical exam
- Hematology and blood chemistry
- Demographic data
- Medical history
- Concomittant Medication
- Evaluation Scales
    - Processing Speed Index
    - Stroop Color-Word Test
    - Trail Making Test
    - Digit Span
    - Montreal Cognitive Assessment
    - Hamilton Anxiety Rating Scale
    - Hamilton Rating Scale for Depression
    - One Touch Stockings of Cambridge
    - Multitasking Test
    - Reaction Time
- ET

- qEEG

Treatment cycles

Each patient shall receive three cycles of treatment of 10 infusions on 10 consecutive days

- Study days 31-40,
- Study days 61-70
- Study days 91-100

Visit 1 – Efficacy Evaluation (study day 101)

- Neurological and Physical exam
- Evaluation Scales
    - Processing Speed Index
    - Stroop Color-Word Test
    - Trail Making Test
    - Digit Span
    - Montreal Cognitive Assessment
    - Hamilton Anxiety Rating Scale
    - Hamilton Rating Scale for Depression
    - One Touch Stockings of Cambridge
    - Multitasking Test (MTT)
    - Reaction Time
- ET
- qEEG

Visit 2 – Efficacy Evaluation (study day 180)

- Neurological and Physical exam
- Evaluation Scales
    - Processing Speed Index

- Stroop Color-Word TestTrail Making Test
- Digit Span
- Montreal Cognitive Assessment (
- Hamilton Anxiety Rating Scale
- Hamilton Rating Scale for Depression
- One Touch Stockings of Cambridge
- Multitasking Test (MTT)
- Reaction Time

- ET
- qEEG

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/ rTMS sessions actually administered to each patient will be calculated as the percentage of the total number of IV infusions/rTMS sessions planned per protocol and will provide a measure of treatment compliance.

11.3. Risk assessment and Precautionary Measures

Both the investigational medicinal product and rTMS are in clinical use for many years and have 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: **04 / 2018**

Study/Treatment end: **12 / 2020**

13. STATISTICS

13.1. 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 analyses 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.

13.2. 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.

13.3. Primary Objective

It is the primary objective of this clinical study to assess the efficacy of the combining rTMS and Cerebrolysin treatment versus CRB alone, upon a battery of co-primary neurocognitive outcomes at 3 and 6 months post TBI.

13.4. Primary Efficacy Criteria

13.4.1. Justification for Multidimensional Approach

In the last 30 years, no TBI trial with a traditional design on neuroprotective agents showed a significant treatment effect in moderate to severe TBI (Maas, Roozenbeek, 2010). Thus, the challenge to demonstrate benefit of a novel treatment concepts in TBI is great, but the rewards are regarded as correspondingly high (Maas, Roozenbeek, 2010).

Almost all failed studies used a single measure approach. Nevertheless, outcome after TBI is by definition multidimensional, including neurophysical disabilities, disturbances in mental functioning (e.g., cognitive and executive functioning), and consequential problems in social reintegration.

Thus, in agreement with current recommendations, a multidimensional approach for outcome assessment and classification will be chosen for the present study.

An ensemble of appropriate single efficacy criteria shall be tested by a multivariate, directional test approach, reflecting the “global status of patients in TBI” (Bagiella, 2010), while simultaneously combining two points in time in the sense of a ‘repeated measures design’.

13.4.2. Defined Efficacy Ensemble

The following ensemble of appropriate single efficacy criteria shall be tested by a multivariate, directional test approach, reflecting the global status of patients in TBI after 3 and 6 months:

Multivariate Efficacy Ensemble

1. Processing Speed Index, Changes from Baseline, LPCF, ITT
2. Stroop Color-Word Test, Changes from Baseline, LPCF, ITT
3. Trail Making Test, Changes from Baseline, LPCF, ITT
4. Digit Span, Changes from Baseline, LPCF, ITT
5. Montreal Cognitive Assessment, Changes from Baseline, LPCF, ITT
6. Stockings of Cambridge (CANTAB) , Changes from Baseline, LPCF, ITT
7. Reaction Time (CANTAB) , Changes from Baseline, LPCF, ITT
8. Hamilton Rating Scale for Depression, Changes from Baseline, LPCF, ITT
9. Hamilton Anxiety Rating Scale, Changes from Baseline, LPCF, ITT

13.5. Secondary Objectives

To assess the single efficacy criteria at 3 and 6 months post TBI.

To test ET and qEEG parameters as biomarkers of cognitive dysfunction at 3 and 6 months post TBI.

To test ET and qEEG as treatment monitoring tools at 3 and 6 months post TBI.

To check assay sensitivity for the primary objective (rTMS + CRB versus CRB alone) by comparing CRB alone versus PLC.

To assess the safety of rTMS administrated starting with one month after TBI.

13.6. Secondary Variables

1. Eye tracking parameters
2. Quantitative EEG parameters
3. Safety Parameters (laboratory values, adverse events)

13.7. Level of Significance

The multiple level alpha is set to α = 0.05, one-sided, according to current recommendations for randomized phase II trials (Rubinstein 2009; Sumithra 2010; Manish 2011).

13.8. Multiplicity

Multiplicity regarding multiple primary outcome measures at two points in time is controlled by the chosen correlation-sensitive, multivariate test procedure (Wei-Lachin procedure, see also section 13.10).

13.9. Sample Size Calculation

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

(a) One-sided type I error defined as alpha = 0.05 (multiple level alpha, see section 13.7)

(b) Type II error defined as β = 0.2 (Testpower 80%)

(c) Design alternative effect size: Mann-Whitney statistic (MW) = 0.64 (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 = 0.64 means Cohen's d = 0.5)

(d) Estimated correlations among the single outcome scales included in the global statistics ρ = 0.4)

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, 1989; 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 13.10, 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 30 patients per group (including 10% enhancement for usual „ambiguities“, e.g., dropouts). With this sample size a „medium-sized“ group difference (MW = 0.64) with regard to the multivariate outcome ensemble at month 3 and 6 can be detected with a power of 80%.

13.10. 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 scales used in TBI. Furthermore, data types can be of different nature (binary, ordinal, continuous). Thus, a non-parametric assessment of treatment effects independent of data type and distribution should be chosen as the primary analysis method.

The 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 a directional test which is most efficient in the case of known direction for superiority.

The procedure is described by Wei and Lachin (1984) and Lachin (1992). Practical examples are given in modern textbooks on multiple testing problems(see e.g., Dimitrenko, 2010). 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 TBI studies.

According to the ICH Guideline E9 (ICH Topic E9, Statistical Principles for Clinical Trials, Step 4, Consensus guideline, 5 February 1998, CPMP/ICH/363/96) the results will be given as P-values as well as effect size measures with their confidence intervals (Mann-Whitney statistic as corresponding effects size measure of the Wilcoxon-Mann-Whitney test), so that the direction and quantity of the treatment effects are determined with their precision.The Mann-Whitney statistic is the most valuable effects size measure for the Wilcoxon-Mann-Whitney test because it is appropriate where the Hodges-Lehmann shift parameter is no longer valid. Furthermore, the Mann-Whitney effects size measure is appropriate for continuous, ordinal and binary data at the same time and represents an ideal effects size measure for multiple outcomes. Incidentally, the 25th Anniversary of the journal Statistics in Medicine dedicated a whole issue to papers about the Mann-Whitney statistic (D'Agostino, 2006).

The Mann-Whitney effects size measure (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, defined in statistical shortcut: 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:

$$H_0: \quad MW_{TC} \leq 0.50$$

$$H_A: \quad MW_{TC} > 0.50$$

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

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 ITT 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).

13.11. Exploratory Analyses

All primary and secondary efficacy criteria will be analyze 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.

Analysis procedures for Eye tracking parameters and Quantitative EEG parameters will be described in the Statistical Analysis Plan.

13.12. Accounting for missing data

13.12.1. Missing Data Problems – General Considerations for a TBI Study

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.

13.12.2. 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 (TBI-related neurological reason) [describe reason]

3 = not completed (different reasons, not TBI related) [describe reason]

13.12.3. 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.

13.12.4. 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).

13.13. Definition of study population

13.13.1. 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.

13.13.2. 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.

13.13.3. ITT Population (Full Analysis Set)

ITT 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 (“modified” ITT). This way ITT is defined in the sense of the “full analysis set” according to ICH E9 § 5.2. (“Analysis Sets”) . ITT population will be used for all efficacy analyses.

13.13.4. 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 ITT evaluation and who additionally do not show major protocol deviations. As noted in section 13.10, the supportive analysis by means of the per-protocol set will be regarded as of equal scientific importance as the ITT analysis, since it most closely reflects the scientific model underlying the protocol (see ICH E9, section 5.2.2).

13.14. Homogeneity Analyses (Exploratory Interpretation)

Homogeneity analyses for baseline shall be performed based on the ITT 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.

13.15. 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.

13.16. 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, ITT, 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.

13.17. 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 Sponsor and / or authorised representatives of the Sponsor 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 Sponsor 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 Sponsors 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.

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.

16.4. Modification of Protocol

The Investigator or the Sponsor 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 Sponsor and the Principal Investigator. Necessary protocol amendments will be submitted to the appropriate IECs.

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 should 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 Sponsor. 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 Sponsor 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

The Sponsor and Investigator shall agree on the final study report. The latter is to be signed by the Investigator and the investigating physicians involved.

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 Sponsor and not previously published) is considered confidential and shall remain the sole property of the Sponsor. The Investigator agrees not to use it for other purposes without the Sponsor’s written consent.

It is understood by the Investigator that the Sponsor 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 Sponsor with complete test results and all data developed during this study.

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19. 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:

20.03.2018

Signature: