OBSERVATION PLAN

C-REGS 2

Cerebrolysin **REG**istry **S**tudy in **S**troke

A registry study to observe clinical practices, safety and effectiveness of routine use of Cerebrolysin in the treatment of patients with moderate to severe neurological deficits after acute ischaemic stroke

STUDY IDENTIFICATION №: EVER-AT-0717

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Version Final 3.3
01.03.2021

This observation plan has been written in accordance with the Declaration of Helsinki and applicable local guidelines.

SPONSOR:
EVER Neuro Pharma GmbH
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Austria

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Version Tracker

Final Version before Enrollment Start: Version 3.1 from October 18, 2017

First Patient First Visit (FPFV): April 25, 2018

Changes after First Patient First Visit (FPFV)

Version 3.2 from July 10, 2018

- Pharmaeconomics survey added for Day 21 and Day 90 (EQ-5D-5L, Cost Questionnaire)

Version 3.3 from March 01, 2021

- Timelines adapted due to Covid-related delayed recruitment
- Initiation of the Stage II of the pre-planned Two-Stage Procedure

  Specifications for Scale Training amended

1. Synopsis

Title	**C-REGS 2** **C**erebrolysin **REG**istry **S**tudy in **S**troke A registry study to observe clinical practices, safety and efficiency of routine use of Cerebrolysin in the treatment of patients with moderate to severe neurological deficits after acute ischaemic stroke
Name of finished product	Cerebrolysin
Name of active substance	Cerebrolysin Concentrate
Coordinating investigator	Univ. Prof. Dr. Michael Brainin
Number of sites & countries	Approx. 50 sites in Europe and Asia and (see Appendix 1)
Indication	Acute Ischemic Stroke with moderate to severe neurological deficits
Study Design	Non-interventional, controlled, open-label, prospective, multicenter, restricted cohort observational registry study
Study groups	All patients receive acute stroke care according to local treatment standards, not amended or influenced by the study: **Cerebrolysin Group:** Patients who are treated with Cerebrolysin; dosage, frequency and duration follow local clinical practice in accordance with terms of the local marketing authorization (see Appendix 2) **Control group:** Patients who are not treated with Cerebrolysin
Study timelines	Patient recruitment Q1/2018 – Q1/2021 – Stage I Patient recruitment Q1/2021 – Q4/2023 – Stage II Definition “End of study”: Database closure
Study Duration	Patients are followed over a maximum of 100 days

Sample Size	Approx. 2000 patients within the framework of a two-stage procedure according to Bauer-Köhne. Stage I will be completed after enrollment of approx. 670 patients. Sample size is statistically justified.
Study objectives	Investigation of clinical practices, safety and effectiveness of Cerebrolysin in routine treatment of patients with moderate to severe neurological deficits after acute ischemic stroke
Documented items	**Baseline:** • Patient data (age, gender, ethnicity) • Inclusion/exclusion criteria • Patient logistics • Risk factors • NIHSS • Evidence of dementia (IQCODE) **Treatment:** • Acute interventions • Neurorehabilitation • Cerebrolysin treatment • Other treatments (during hospital stay) **Discharge:** • Patient logistics • Stroke diagnosis (confirmation) • NIHSS • mRS • Neurorehabilitation **Day 21±4:** • NIHSS • mRS **Day 90±10:** • Patient logistics • NIHSS • mRS • Cognitive status (MoCA) • New event (within three months) • Neurorehabilitation **Death:** • Date/time • Cause **Adverse events:**

	• Date/time • Relationship/Seriousness/Outcome **Pharmaeconomics survey (Day 21±4, Day 90±10, Discharge):** • EQ-5D-5L (EuroQol) • Cost Questionnaire
Eligibility criteria	**Observation criteria:** • Signed Informed Consent • Clinical diagnosis of acute ischemic stroke confirmed by imaging • Moderate to severe neurological deficits with NIH Stroke Scale (NIHSS) 8 to 15, both inclusive • No prior stroke • No prior disability • Patient's independence prior to stroke onset (pre-morbid mRS of 0 or 1) • Reasonable expectation of successful follow-up (max. 100 days)
Statistical Methods	**Sample Size Calculation:** Nonparametric sample size calculation was performed to allow detection of "small" group differences in the ordinal comparative effectiveness evaluation with 90% power. The study will use a two-stage adaptive design according to Bauer-Köhne. The total sample size including compensation for 'usual ambiguities' (dropouts, etc.) results in approx. 2000 patients to be enrolled (stage I and stage II). The first stage will enroll approximately 670 patients ($r_{subsample\ I}$ = 0.3). If there is no rejection after stage I analysis due to success or futility, the trial may continue to stage II. **Bias minimizing measures:** • Enrolment Bias: In order to minimize enrolment bias, the patient groups will be standardized using multilevel stratification procedures in combination with a 'restricted cohort' design. The respective risk factors have been identified from published research results on predictors of stroke outcome, allowing appropriate control for confounders. The pre-specified strategy follows the recommendations of the *Principles for Good Research on Comparative Effectiveness (GRACE)*. • Quality assurance The study shall be conducted in a manner fully consistent with good clinical practice. Data will be captured using an eCRF-system with quality assurance performed by edit checks and frontline risk-based control. In addition, and in order to comply with recent calls for high-quality non-interventional comparative effectiveness research, 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 according to the

	most recent requirements of the ICH E6 *Guideline for Good Clinical Practice* (GCP, Amendment R2, July 2015), the FDA Guidance for Industry on a *Risk-based Approach to Monitoring*, and the EMA reflection-paper on *risk-based quality management in clinical trials*.
	
	• Other sources of bias:
	
	Other aspects of care than administration of study drug may vary between the study groups. Analyses will consider these potential sources of variation by appropriate sensitivity analyses.
	
	Effectiveness analysis:
	
	• Primary effectiveness analysis:
	  ○ Ordinal modified Rankin Scale (mRS) at 3 months after stroke onset
	• Secondary effectiveness analyses include:
	  1. Ordinal NIH Stroke Scale (NIHSS) at 21 days and 3 months after stroke onset
	  2. Ordinal modified Rankin Scale (mRS) at 21 days after stroke onset
	  3. Proportion of patients with excellent recovery (mRS score 0-1) at 3 months after stroke onset
	  4. Proportion of patients with functional independence (mRS score 0-2) at 3 months after stroke onset
	  5. Ordinal MoCA at 3 months after stroke
	
	Details of the planned case-mix adjustment using multilevel standardization methods and of the statistical analysis procedures will be provided in the statistical analysis plan that will be issued before enrolment of the first patients. Any subsequent changes will be fully documented with audit trail.
	
	Safety analysis:
	
	• Mortality
	• AEs, ADRs, SAEs, SADRs
	• SUSARs to Cerebrolysin
	
	Optional: Pharmaeconomics survey:
	
	• EQ-5D-5L (EuroQol)
	• Cost Questionnaire

2. Summary and Justification

Stroke is a devastating disease and one of the primary causes for death and long-term morbidity imposing a heavy burden on patients, relatives and the health care system. Except for fibrinolytic therapy, which is only possible in a minor fraction of patients, there is no widely approved medication for the treatment of acute stroke.
Cerebrolysin has been approved for the treatment of stroke in over 45 countries worldwide.
Since the approval of Cerebrolysin, stroke therapy has evolved, namely, with improved overall care, stroke units, more targeted rehabilitation, and the increasing availability of fibrinolytic therapy (rtPA, Actilyse) in specialized centers throughout the world. More recently, interventional therapies with various thrombus retrievers have emerged.
In addition, the Cerebrolysin treatment in stroke has evolved with different time windows, dosages and lengths of therapy being given in a pragmatic way by physicians within the specification of Product Characteristics for Cerebrolysin (SPC).

The main aim of this study is to systematically record Cerebrolysin treatment modalities and concomitant medication, according to local standards, in patients with moderate to severe neurological deficits after acute ischemic stroke and to assess the impact of these parameters on therapy outcome during early rehabilitation (day 21) and on day 90.

Besides this, the effectiveness and safety of Cerebrolysin therapy is monitored against the background of the now established and evolving stroke therapies (rtPA, thrombectomy). Furthermore, effectiveness and safety of Cerebrolysin will be evaluated according to pre-existing diseases, to concomitant medication and to applied rehabilitative actions. In the concomitant control group these therapies alone or in combination will be compared to the addition of Cerebrolysin in these patients. Of interest is also the treatment in stroke units, with rtPA and systematic rehabilitation until day 21 and day 90.

An open observational treatment design has been chosen to collect data to capture the therapies as applied in real clinical practice. In order to minimize enrolment bias, the patient groups will be standardized using multilevel stratification procedures in combination with a ‘restricted cohort’ design. The respective risk factors have been identified from published research results on predictors of stroke outcome, allowing appropriate control for confounders. The pre-specified strategy follows the recommendations of the Principles for Good Research on Comparative Effectiveness (GRACE).
In order to make valid conclusions, rigorous data methodologies shall be applied, and the study shall be conducted in a manner fully consistent with good clinical practice[1].
An international Steering Committee shall supervise the activities of the different working groups in order to assure an unbiased conduct and analysis of the study.

3. Introduction

3.1 Background information

Cerebrolysin is a neuropeptide preparation with marketing authorization for the treatment of cerebrovascular disorders and neurodegenerative disease for many years worldwide. Since its first approval stroke therapy has evolved and new treatment concepts have been implemented. In addition, Cerebrolysin treatment in stroke has evolved with different time windows, dosages and lengths of therapy being given in a pragmatic way by physicians. The main aim of this study is to capture these variables of the Cerebrolysin treatment and its comedication in the group of patients with moderate to severe neurological deficits after acute ischemic stroke in order to give guidance to further research.
C-REGS 2 is an international, non-interventional, prospective registry study to observe clinical practices of routine use of Cerebrolysin in patients with moderate to severe neurological deficits after acute ischemic stroke in a controlled and open-labelled manner. All patients receive acute stroke care according to local treatment standards, which will not be amended or influenced by the study in any way. To evaluate the safety and effectiveness of Cerebrolysin in routine practice the outcome of Cerebrolysin-treated patients are compared with control group patients, who do not receive Cerebrolysin

3.2 Study rationale

The reason to perform this study is to systematically record Cerebrolysin treatment modalitites and concomitant medication in patients with moderate to severe neurological deficits after acute ischemic stroke and to assess the impact of these parameters on therapy outcome in particular on early benefit for the patients and on day 90.

4. Study Objectives

The objective of this registry study is to observe clinical practices, safety and effectiveness of routine use of Cerebrolysin in the treatment of patients with moderate to severe neurological deficits after acute ischemic stroke. A systematic record of Cerebrolysin treatment modalities and concomitant medication in stroke patients with moderate to severe neurological deficits will be performed and the impact of these parameters on therapy outcome on day 21 and day 90 will be assessed.

4.1 Documented items:

Baseline:

- Patient data (age, gender, ethnicity)
- Observation criteria
- Patient logistics
- Risk factors
- NIHSS
- Evidence of dementia (IQCODE)

Treatment:

- Acute interventions

- Neurorehabilitation
- Cerebrolysin treatment
- Other treatments (during hospital stay)

Discharge:

- Patient logistics
- Stroke diagnosis (confirmation)
- NIHSS
- mRS
- Neurorehabilitation

Day 21±4:

- NIHSS
- mRS

Day 90±10:

- Patient logistics
- NIHSS
- mRS
- Cognitive status (MoCA)
- New event (within three months)
- Neurorehabilitation

Death:

- Date/time
- Cause

Adverse events:

- Date/time
- Relationship/Seriousness/Outcome

Optional: Pharmaeconomics survey (Day 21±4, Day 90±10, Discharge):

- EQ-5D-5L (EuroQol)
- Cost Questionnaire

5. Study Design

Non-interventional, controlled, open-label, prospective, multicentre, restricted cohort observational registry study.

6. Selection and withdrawal of patients

6.1 Informed Consent

In obtaining and documenting informed consent, the investigator will comply with the applicable regulatory requirement(s), and will adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki.
Accordingly, prior to a subject's participation in the trial, the Informed Consent form has to be signed and personally dated by the subject and the health care professional conducting the Informed Consent process.
Surrogate consent may be obtained according to the local regulations for not competent patients. However, personal consent will be obtained from each patient after recovering competence.
The Investigator will explain the nature, purpose and risks of the study and provide the patient/legally authorized representative with a copy of the patient information sheet in the regional language. The patient/legally acceptable representative will be given ample time to consider the study's implications before deciding whether to participate.

6.2 Patient Eligibility Criteria

Criteria for observed patient population:

- Signed Informed Consent
- Clinical diagnosis of acute ischemic stroke, confirmed by imaging
- Moderate to severe neurological deficits (observation window: NIHSS 8-15)
- No prior stroke
- No prior disability
- Patient's independence prior to stroke onset (pre-morbid mRS of 0 or 1)
- Reasonable expectation of successful follow-up (max. 100 days)

6.3 Stopping and Discontinuation

Patients will be advised in the Informed Consent Forms that they have the right to withdraw from the study at any time without prejudice.

6.4 Randomization, Blinding and Unblinding

This study has a non-interventional, observational character. The therapeutic strategy follows standard hospital practices and is not determined by the observational plan. Thus, randomization and blinding/unblinding procedures are not applicable for this study.

In order to overcome the enrolment bias, treatment groups will be standardized using multilevel stratification procedures in combination with a 'restricted cohort' design. Case-mix standardization methods will be *a priori* defined in the statistical analysis plan before enrolment of the first patients.

7. Investigational Product

Cerebrolysin has marketing authorization in the countries participating in C-REGS 2.
C-REGS 2 is an observational study, thus treatment of patients follows standard hospital practices and is clearly separated from the decision to include a patient in the study.
Thus, Cerebrolysin is purchased from the local markets by the hospitals and is not provided by the sponsor.

7.1 Name and Description of the Investigational Product

Cerebrolysin is a neuropeptide preparation produced by a standardized enzymatic breakdown of purified, lipid-free porcine brain proteins. It consists of low molecular weight neuropeptides (<10 kDa) and free amino acids. Cerebrolysin has been used for the treatment of cerebrovascular and neurodegenerative diseases like stroke, dementia and traumatic brain injury for many years.
For detailed information on the product please refer to the SPC in Appendix 2.

7.2 Dosage, Formulations and Administration

Dosage, frequency, duration and mode of administration of Cerebrolysin follow the local hospital practice in accordance with the terms of the local marketing authorization (see Appendix 2) and is not amended or influenced by the study.
Prescribed Cerebrolysin will be used as solution for injection/concentrate for solution for infusion.

8. Concomitant Therapy

Concomitant medication is not restricted or influenced by the study and will be documented in the eCRF.

9. Assessment of Effectiveness

No additional diagnostic, therapeutic or monitoring procedures other than those used according to local practice will be applied to the patients included in the study.

Tests selected to assess effectiveness have been chosen in accordance with the recommendations of various stroke guidelines:

The **modified Rankin Scale** (mRS) has been widely used for many years in clinical practice to measure global functional outcome.

The **National Institutes of Health Stroke Scale** (NIHSS) is described as the standard for the assessement in acute stroke in the “Guidelines for the early management of patients with acute ischemic stroke” by the American Heart Association/ American Stroke Association, the ESO Guidelines for “Management of Ischaemic Stroke and Transient Ischaemic Attack” and in the Austrian Neurology Society (ÖGN-ÖGSF) guideline (Chapter 12)[2]. The NIHSS reflects neurological impairment, the clinical domain in which early effects of acute stroke therapies are likely to be most marked. Recent research showed that the NIHSS in fact is most sensitive for early points in time[3]. Furthermore, it is less influenced by extraneous

factors, improving sensitivity to acute treatment effects.
The assessment of cognitive deficits following stroke is recommended by several guidelines (ESO Guidelines, Austrian Neurology Society[4] and the DEGAM Stroke Guideline nr. 8). The DEGAM guideline mentions the Mini-Mental State Examination (MMSE) for assessment. However, compared to the MMSE the **Montreal Cognitive Assessment** (MoCA) was shown to be more sensitive for the detection of cognitive impairment after acute stroke[5] and is recommended by the "National clinical guideline for Stroke"[6].

To assess premorbid cognitive dementia the **Informant Questionnaire on Cognitive Decline in the Elderly** (IQCODE) is described as a valuable tool[7], which has been widely adopted in clinical practice[8]. The IQCODE is done as a prerequisite for the MoCA assessment in order to identify patients with cognitive dysfunction prior to stroke.

9.1 Definitions of Assessments

mRS
The Modified Rankin Scale[9] is a functional global outcome scale measuring the level of disability after a stroke. The test can be answered in approximately 5-10 minutes. It is a 7-point ordinal scale with a score of 0 indicative of no residual symptoms and the worst possible score of 6, which is assigned in case of death.

The mRS assessment is performed using the Rankin Focused Assessment (RFA)[10],[11] in order to provide unambiguous operationalization and ensure consistent score determination across raters.

NIHSS
The National Institutes of Health Stroke Scale is a systematic assessment tool that provides a quantitative measure of stroke-related neurological deficits. A total of 15 items evaluate the level of consciousness, language, neglect, visual-field loss, extraocular movement, motor strength, ataxia, dysarthria, and sensory loss.

A trained observer rates the patient's ability to answer questions and perform activities. Ratings for each item are scored with 3 to 5 grades with 0 as normal, and there is an allowance for untestable items. The single patient assessment requires less than 10 minutes.

MoCA
The Montreal Cognitive Assesment is a screening tool for mild cognitive dysfunction, which takes approximately 10 minutes[12]. It assesses various cognitive domains: attention and concentration, executive function, memory, language, visuoconstructional skills, conceptual thinking, calculations and orientation. The possible maximum score is 30 points and 26 or above is considered normal.

IQCODE
The Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE-short version)

screening tool is a questionnaire of 16 questions designed to assess cognitive decline and dementia[7,8]. It is filled out by a person who has known the patient for 10 or more years.

9.2 Mandatory Training of Scales

For the NIHSS, the mRS and the MoCA a mandatory training has to be performed by all personnel using the scales. For the MoCA training is provided on the MoCA homepage (mocatest.org). For the NIHSS training videos from the American Heart Association are used in combination with a local trainer. For the mRS the Rankin Focused Assessment – Ambulation will be used to provide unambiguous operationalization and ensure consistent score determination across raters. Training videos will be shown to train the interview technique. . A retraining will be done every two years for mRS and NIHSS.

Rating has to be performed by site personnel that is independent from treatment administration.

9.3 Documented parameters

Acute stroke patients admitted to hospital are stabilized and receive regular care. It is important to note that the treatment of patients will not be influenced in any way by the study. Patients complying with the observation criteria are eligible for study participation. Upon giving informed consent patient's data are recoded in the eCRF.

The following data are entered in the eCRF:

Assessment	Source document
Informed consent given	Informed consent form
Baseline	
Patient data (age, gender, ethnicity)	Patient file
Observation criteria	Patient file
Patient logistics	Patient file
Confirmation of observation criteria	Patient file
Risk factors	Patient file
NIHSS	Rating sheet
Evidence of dementia (IQCODE)	Patient file, Rating sheet
Treatment	
Acute interventions	Patient file
Rehabilitation	Patient file
Cerebrolysin treatment	Patient file
Other treatments (during hospital stay)	Patient file
Discharge	
Patient logistics	Patient file
Stroke diagnosis (confirmation)	Patient file
Rehabilitation	Patient file
Day 21±4	
Type of stroke (TOAST)	Patient file

NIHSS	Rating sheet
mRS	Rating sheet
Rehabilitation	Patient file
Pharmaeconomic survey*	Rating Sheet
Day 90±10	
Patient logistics	Patient file
mRS	Rating sheet
New event (within three months)	Patient file
Cognitive status (MoCA)	Rating sheet
NIHSS	Patient file
Rehabilitation	Patient file
Pharmaeconomic survey*	Rating Sheet
Death	
Date/ time	Patient file
Cause	Patient file
Adverse events	
Date/time	Patient file
Relationship/Seriousness/Outcome	Patient file

* optional

10. Assessing and reporting of adverse events

Throughout the course of this non-interventional study particular attention is paid to the Adverse Events and Adverse Drug Reactions mentioned below.

10.1 Definitions

10.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a patient 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 a pharmaceutical product, whether or not related.

10.1.2 Adverse Drug Reaction (ADR)

An ADR is a response to a medicinal product which is noxious and unintended and arises from the use of a medicinal product either within or outside the terms of the marketing authorization (including overdose, off-label use, misuse, abuse and medication errors).

An AE judged as having a “reasonable causal relationship” to the pharmaceutical product qualifies as ADR. This corresponds to the categories “possible”, “probable” and “definite”:

Causality code	Definition
Not assessable	A report suggesting an Adverse Event, which cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified.
Not related	A report suggesting an Adverse Event which does not follow a reasonable temporal sequence from administration of the drug and is clearly related to other factors, such as clinical state, therapeutic intervention or concomitant therapy.
Unlikely	A clinical event, including laboratory test abnormality, with a temporal relationship, which makes a causal relationship improbable, and in which other drugs / treatments, chemicals or underlying disease(s) provide plausible explanations.
Possible	A clinical event, including laboratory test abnormality, with a reasonable temporal relationship to administration of the drug / treatment, but which also could be explained by concomitant diseases or other drugs / treatments or chemicals.
Probable	A clinical event, including laboratory test abnormality, with a reasonable temporal relationship to administration of the drug / treatment, unlikely to be attributable to concomitant disease(s) or other drugs / treatments or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfil this definition.
Definite	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to study treatment and which cannot be explained by concomitant disease(s), other drugs / treatments or chemicals. The response to withdrawal of the treatment (dechallenge) should be clinically plausible. The event must be unambiguously either pharmacologically or as phenomenon, using in satisfactory rechallenge procedures if necessary.

10.1.3 Serious Adverse Event (SAE) / Serious Adverse Drug Reaction (SADR)

An AE / ADR is considered serious if it

- results in death
  - **Please note:** Death is not an SAE / SADR, it is the outcome of an SAE / SADR. The SAE/SADR to be reported comprehensively is the medical condition leading to death, e.g. underlying disease, accident.

- is life threatening
  - **Please note:** Life threatening means that a 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.
- requires additional inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability / incapacity
- is a congenital anomaly / birth defect
- is other medically significant

In other situations medical judgement should be exercized in deciding whether an AE / ADR is serious:

Important AEs / ADRs that are not immediately life-threatening or do not result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

10.2 Reporting of ADRs

Depending on applicable regulatory requirements serious and non-serious adverse drug reactions might qualify for expedited reporting to Health Authorities (HAs). In order to comply with these regulatory requirements, it is vitally important that the Investigator enters **within 24 hours** any Adverse Event in the corresponding sheet in the eCRF. This will create an immediate automated email to drugsafety@everpharma.com informing the study safety officer

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The Sponsor will notify the competent authorities of ADRs related to Cerebrolysin in line with the Guideline on Good Pharmacovigilance Practice (GVP) and in line with applicable local regulatory requirements.

The treating physician will support the Sponsor in notifying the competent authorities in some non-European countries (further details are defined in the respective site contract).

ADRs related to any other medication will be notified to the competent authorities by the treating physician.

Serious ADRs will be notified to competent authorities within 15 days and non-serious ADRs will be notified to competent authorities within 90 days. Applicable local regulatory requirements may differ and will be adhered to.

10.3 AE / ADR follow-up

AEs / ADRs will be followed up as necessary to obtain supplementary detailed information significant for the scientific evaluation.

11. Statistical Methods

11.1 General Principles

This is a registry-based non-interventional study observing safety and effectiveness of treatment after acute ischaemic stroke under real-life practice conditions.

A non-interventional study offers useful insight into safety, effectiveness and tolerability in day-to-day practice. It is a "pragmatic" approach as opposed to the "explanatory" approach of a randomized clinical trial (RCT) with its highly controlled conditions.

However, selection of patients for exposure to treatment based on clinical features and physician preference instead of random allocation inevitably introduces opportunities for bias and confounding. According to the principles of *Good Research for Comparative Effectiveness Research* (GRACE)[13], and in line with the HTA recommendations for non-randomized studies[14], appropriate control of confounding variables together with rigorous pre-specification of analytical techniques is one of the primary requirements for high quality effectiveness research.

11.2 Outcomes and case-mix standardization

Ordinal analysis of the modified Rankin scale (mRS) at 3 months after stroke onset is chosen as clinically relevant primary endpoint for final treatment effects. Leading secondary endpoint is the NIHSS score on day 21 and 90.

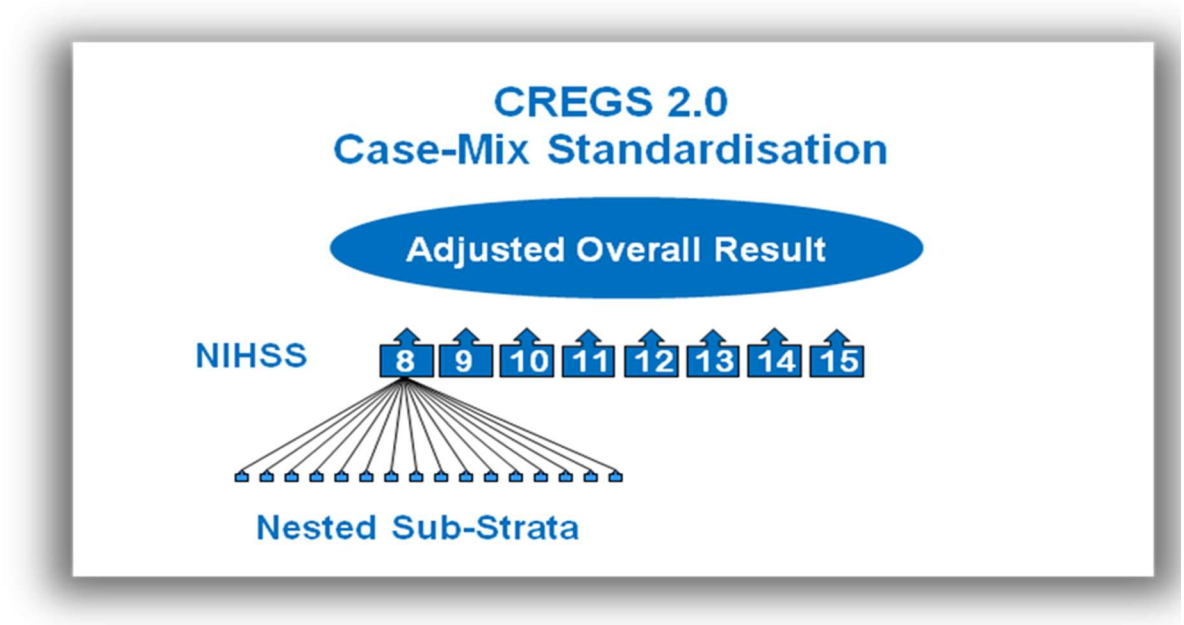
In order to minimize enrolment bias, the patient groups will be standardized using nonparametric multilevel stratification procedures in combination with a ‘restricted cohort’ design. The respective risk factors have been identified from previous research results on NIHSS predictor variables, allowing appropriate control for confounders of outcome after acute ischemic stroke[15,16,17]. The pre-specified case-mix standardization strategy follows the recommendations of the GRACE Principles for Good Research on Comparative Effectiveness[13,18] and is based on multilevel control for potential confounders (‘like-with-like’ comparison).

The top level case-mix standardization will be based on the NIHSS baseline score as one of the strongest predictors for outcome after stroke[15,16,17]. The top level control will be

performed by implementing stratification per NIHSS score unit with subsequent meta-analytic pooling of strata (i.e., comparing groups within identical baseline NIHSS score). The eligibility restriction to NIHSS 8-15 allows full stratification for each possible baseline score.

The second level case-mix standardization is performed for control of remaining important confounders. It is implemented within each of the top level NIHSS strata by means of nested sub-strata and subsequent adjustment by means of the Cochran-Mantel-Haenszel pooling procedure (also known as the van-Elteren procedure[19]). This procedure allows unbiased adjustment of ordinal data also in the presence of very low sample sizes within single strata.

The combined, adjusted overall result of the multilevel stratification procedure will be provided with associated effect size and confidence interval in order to allow evidence-based effectiveness evaluations.



The advantage of the chosen multilevel case-mix standardization as compared to other, model-based approaches such as regression models is that any assumption about the nature of risk-outcome relation is avoided, allowing true like-to-like comparisons. Furthermore, potential drawbacks of other procedures, as the model-based propensity score matching with its risk of bias due to incomplete matching, are reduced.

In addition to the multilevel case-mix standardization, a specific method used to strengthen observational studies is introduced for further control of confounders: the 'restricted cohort' design[20,21]. It adapts basic eligibility principles of the design of randomized, controlled trials to the design of an observational study. As defined above, patients are only eligible for this registry trial with clinical diagnosis of acute ischemic stroke confirmed by imaging, with moderate to severe neurological deficits (NIHSS inclusion window: 8 to 15)[16], and without prior stroke or prior disability[15]. The restricted eligibility definition is chosen to further reduce potential for confounding[13,18,20,21].

Full details of the planned case-mix standardization methods and of the statistical analysis procedures will be provided in the statistical analysis plan that will be issued before enrolment of the first patients. Any subsequent changes will be fully documented with audit trail.

11.3 Two-Stage Procedure

The two-stage adaptive procedure of Bauer P and Köhne K (1994) is chosen as the sequential method.

The two-stage procedure based on Fisher's combination test (Bauer and Köhne)[33] shows only a negligible loss in test power as compared to a fixed sample size study but allows early stopping due to success or failure[33,22]. Furthermore, assumptions for sample size calculation can be rechecked after stage I. The same applies to design modifications within the framework of the adaptive approach although this is not the rationale for introducing the two-stage procedure in this study. The formal Bauer-Köhne *futility* benchmark is set for this study to $\alpha_0 = 0.3$. It is important to note that this benchmark is taking into account the limited number of available patients for a possible stage II due to the restricted cohort design.

With a global multiple level alpha = 0.05 two-sided, and defined futility level of $\alpha_0 = 0.3$ the following decision structure will be formally established (p_1 = P-value of stage I, p_2 = P-value of stage II):

Decision Structure for Stage I results (two-sided)

$p_1 \geq \alpha_0 = 0.3$	: stop because of futility
$p_1 \in (0.0299; 0.3)$	: continue with stage II
$p_1 \leq \alpha_1 = 0.0299$	: stop with success (rejection of H_0)

Decision Structure for Stage II results (two-sided)

$p_1 p_2 > \alpha_c = 0.0087$	: stop because of futility
$p_1 p_2 \leq \alpha_c$	: rejection of H_o (proof of efficacy)

11.4 Sample Size

As this is an observational study, the relations among effect size, sample size and power estimates are indicative only. However, some justification is needed to support the planning of the number of centers and duration of enrolment period.

According to the recommendations of the *Principles for Good Research on Comparative*

Effectiveness (GRACE)[13]*,* the study size intended for the observational trial "should be described including a description of how that size was determined, what specific assumptions are being made, and how well these assumptions are supported".

Formal nonparametric sample size calculation was performed to allow detection of "small" group differences in the ordinal comparative effectiveness evaluations with 90% power.

The sample size calculations are based on the following design specifications:

(a) Two-sided type I error defined as alpha = 0.05 (multiple level alpha)

(b) 90% power (1 – beta)

(c) Effect size measure for ordinal scales: Mann-Whitney measure of superiority (MW) [23,24,25,26,27,28,29,30]

(d) Difference to be detected: MW = 0.55 (equivalent to a „small" difference according to Cohen[31,32]

(e) Assumed maximum imbalance between enrolled groups: 1:2

(a) Two-Stage Adpative Design with α_0 = 0.3; Stage I sample size = 30% of total sample size ($r_{subsample\ I}$ = 0.3); power compensation for defined two-stage parameters based on Newton Cotes-algorithm of fifth order[33]

(f) Nonparametric model: stochastic superiority (minimized assumptions)

In the presence of the above assumptions the *calculated* total sample size results in 1745 subjects (including power adjustment for the chosen parameters of the two-stage procedure). For compensation of usual ambiguities (drop outs etc.) the calculated sample size is enhanced by a factor of 1.15 (15%) from 1745 to a total of approx. 2000 subjects. This way, at least 90% power is guaranteed within the framework of the two-stage procedure.

Stage I is completed after enrollment of about 30% of the planned total patients ($r_{subsample\ I}$ = 0.3). For practical purposes the sample size for stage I will be rounded up to 670 patients, i.e., the completion of stage I is defined as recruitment of 670 patients.

Nonparametric sample size calculations within the framework of a Two-Stage procedure (Bauer-Köhne)[33] were based on the model of stochastic superiority and have been performed applying the validated software Nnpar 1.0 and Bauer-Köhne 4.0 from IDV Data Analysis and Study Planning, Krailling/Munich).

12. Access to Source Data/documents

The Investigator will permit study-related monitoring, audits, EC review and regulatory inspections and provide direct access to source documents (see 9.2) and information on any other activities, which are necessary in order to verify that

- the rights and well-being of human subjects are protected
- the reported trial data are accurate, complete, and verifiable from source documents
- the conduct of the trial is in compliance with the currently approved protocol/amendment(s), and with the applicable regulatory requirement(s).

Any party (e.g. domestic and foreign regulatory authorities, the Sponsor and / or authorized representatives of the Sponsor such as monitors and auditors) with direct access to patient data should take all reasonable precautions within the constraints of the applicable regulatory requirements to maintain the confidentiality of patient identities and Sponsor proprietary information.

Access to source data/documents is integral part of the Informed Consent.

13. Quality Control and Quality Assurance

The study shall be conducted in a manner fully consistent with good clinical practice[34].

13.1 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.

13.2 Study Monitoring

Authorized, qualified representatives of the Sponsor might visit investigational sites to verify adherence to observation plan and local legal requirements, to perform source data verification and to assist the Investigator in his study related activities.

13.3 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 the applicable regulatory requirements.

13.4 Inspection

An Inspection is defined as the act by a regulatory authority 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 organization facilities or at any other establishments deemed appropriate by the regulatory authorities.

13.5 Risk-Based Centralized Statistical Monitoring

Data will be captured using an eCRF-system with quality assurance performed by edit checks and frontline risk-based control. In addition, and in order to comply with recent calls for high-quality non-interventional comparative effectiveness research[18], 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 according to the most recent requirements of the ICH E6 *Guideline for Good Clinical Practice* (GCP, Amendment R2, July 2015)[34], the FDA Guidance for Industry on a *Risk-based Approach to Monitoring*[35], and the EMA reflection-paper on *Risk-based Quality Management in Clinical Trials*[36].

14. Pharmaeconomics Survey

Within the framework of the upcoming regulation of the European Parliament and of the Council on health technology assessment[37], a pharmaeconomics survey will be implemented in selected sites by introducing two specific patient-completed questionnaires:

- **EQ-5D-5L (EuroQol)**
- **Cost Questionnaire**

EQ-5DL[38] is a standardised measure of health status developed by the EuroQol Group in order to provide a simple, generic measure of health for clinical and economic appraisal. The EQ-5D-5L consists of the EQ-5D descriptive system and the EQ visual analogue scale (EQ VAS). The descriptive system comprises 5 dimensions (mobility, self-care, usual activities, pain/discomfort, anxiety/depression). Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. The EQ VAS records the respondent's self-rated health on a 20 cm vertical, visual analogue scale with endpoints labelled 'the best health you can imagine' and 'the worst health you can imagine'. This information can be used as a quantitative measure of health as judged by the individual respondents.

The cost questionnaire is a short, 8-section questionnaire that was tailored to extract healthcare resource utilization data. The content of the instrument is based on the CESAR[39] patient costs questionnaire developed by DIRUM. It includes topics like patient transportation, access to nursing, therapy and social services, hospital care, personal (out-of-pocket) expenditure and employment. The questionnaire was adapted in line with the World Health Organization cost effectiveness and strategic planning (WHO-CHOICE) guidelines and the Joint Learning Network Costing Toolkit. This tool will be applied either to patients or their next of kin (when the patient is severely impaired).

The objective of this pharmaeconomics survey is to gain insight into the cost-effectiveness of Cerebrolysin as pharmacological support after stroke comparing to acute stroke care according to local treatment standards. Details of the exploratory analyses of the pharmaeconomics survey will be specified in a separate statistical analysis plan.

15. Signatures

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

Coordinating investigator

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04.03.2021

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Statistician

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Date: Signature:

Study safety officer	Barbara Köth, MD Oberburgau 3 4866 Unterach Austria +43 7665 20555 432 drugsafety@everpharma.com

15/ Mar / 2021 _____
Date: Signature:

Appendices

Appendix 1: Country list (anticipated)

Europe:
Austria
Poland
Romania
Russia
…

Asia:

Philippines
Vietnam
Korea
…

Appendix 2: SPC

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