# CLINICAL STUDY PROTOCOL

## Combined neuropsychological, neurophysiological and psychophysiological evaluation of the effects of N-Pep-12 on neurorecovery in patients after Traumatic Brain Injury (TBI)

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| **Study code** | NPEP-TBI |
| **Protocol number** | FSNANO15012026 |
| **Version** | 01 |
| **Date** | 15.01.2026 |
| **Coordinator** | Foundation for the Study of Nanoneurosciences and Neuroregeneration (FSNN) |

### Confidential information

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This protocol has been drawn up in accordance with the ICH-GCP guidelines and the Declaration of Helsinki, in their updated versions.

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## ABBREVIATIONS AND DEFINITIONS

| | |
|---|---|
| AE | Adverse event |
| SAE | Serious adverse event |
| CRF | Case report form |
| FSNN | Foundation for the Study of Nanoneurosciences and Neuroregeneration |
| GCP | Good Clinical Practice |
| ICH | International Conference on Harmonisation |
| BMI | Body mass index |
| ADR | Adverse drug reaction |
| SADR | Serious adverse drug reaction |
| SUSAR (expected) | Suspected serious adverse reaction, expected |
| SUSAR (unexpected) | Suspected unexpected serious adverse reaction |
| SmPC | Summary of product characteristics |
| APT | Attentional Performance Test |

# 1. PROTOCOL SUMMARY

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| **Coordinator** | FSNN – Foundation for the Study of Nanoneurosciences and Neuroregeneration |
| **Title** | **Combined neuropsychological, neurophysiological and psychophysiological evaluation of the effects of N-Pep-12 on neurorecovery in patients after Traumatic Brain Injury (TBI)** |
| **Study code** | NPEP-TBI |
| **Study site** | Cluj-Napoca County Emergency Clinical Hospital and RoNeuro Institute, Centre for Research and Diagnosis of Neurological Diseases, Cluj-Napoca |
| **Food supplement** | MemoProve/Cebrium |
| **Active substance** | N-PEP-12 |
| **Indication** | Traumatic brain injury |
| **Study characteristics** | Prospective, randomised, controlled, single-blind |
| **Study duration** | Start date: 2026 End date: 2028 |
| **Sample size** | Total: 200<br><br>Group 1 – n-pep 12 – 100<br>Group 2 – control – 100 |
| **Sample dimensioning** | • K=2 experimental treatments will be included in the study.<br>• A significance level of α=0.05 will be used, in combination with the Dunnett correction.<br>• The standard deviations of the response are assumed to be: (σ0,…,σK)=(1,1,1).<br>• The minimum marginal power will be controlled at the level 1−β=0.9 under each of the corresponding least favourable configurations.<br>• The treatment effects will be: δ1=0.5 and δ0=0.<br>• The target allocation for the experimental arms will be identical to that of the control arm.<br>• Consequently, the allocation ratios realised in the experimental arms are: (r1,…,rK)=(1,1).<br>• The maximum family-wise error rate is: 0.05.<br>• The minimum marginal power is: 0.9.<br>• The critical threshold suggested for use together with the multiple-comparison correction is: 0.028.<br>• Adjustment for patients who drop out: 10%<br><br>The total sample size is: N=270. The sample size for each arm is: (n0,…,nK)=(90,90,90).<br><br>*Source: Grayling MJ, Wason JMS (2020) A web application for the design of multi-arm clinical trials. BMC Cancer 20:80. DOI:* |

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| | *10.1186/s12885-020-6525-0* |
| **Randomisation** | 1:1 ratio, 4 blocks, ICH E9 |
| **Case report form** | Electronic, open-source, developed in-house, ICH E6 |
| **Primary objective** | To evaluate the efficacy of daily supplementation for 180 days with 90 mg N-Pep-12 on neurocognitive function and neurological recovery in patients with post-TBI neurocognitive deficit, on day 180 of the study. |
| **Secondary objectives** | To evaluate the efficacy of daily supplementation for 180 days with 90 mg N-Pep-12 on neurocognitive function and neurorecovery in patients with post-TBI cognitive deficit, on days 30 and 90. |
| **Screening variables** | • Medical examination<br>• Demographic data |
| **Primary variables** | • Montreal Cognitive Assessment (MoCA)<br>• Hospital Anxiety and Depression Scale (HADS)<br>• Digit Span (DS)<br>• Color Trial Test (CTT)<br>• Processing Speed Index (PSI)<br>• Rey Auditory Verbal Learning Test (RAVLT) |
| **Secondary variables** | • AE / SAE<br>• EQ-5D-5L<br>Subgroup analysis:<br>• QEEG parameters<br>• Eye Tracking parameters<br>• EQ-5D-5L |
| **Inclusion criteria** | • Patients with mild, moderate or severe traumatic brain injury (TBI) established following a neurological/neurosurgical medical examination.<br>• Patients in the post-acute stage of TBI, at least 30 days after the TBI, with a stable clinical condition.<br>• Presence of residual cognitive and/or affective dysfunction, documented by neuropsychological assessment (MoCA and/or HADS and/or persistent subjective neurocognitive complaints).<br>• Isolated traumatic brain injury (AIS score ≤ 2 in other parts of the body).<br>• Karnofsky performance index prior to the trauma = 100.<br>• Age between 18 and 80 years.<br>• Patient able to give written informed consent for inclusion in the study.<br>• Patient willing and able to comply with the requirements of the protocol for the entire duration of the study.<br>• Women of childbearing potential must have a negative urine pregnancy test and must agree to use adequate |

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| | contraceptive methods.<br>• The patient must have been able to speak, read and write before the accident. |
| **Exclusion criteria** | • Patient with polytrauma (AIS score > 2 in other parts of the body).<br>• Patient with spinal cord lesions.<br>• Major pre-existing and active psychiatric disorder (for example: major depression, schizophrenia, bipolar disorder, dementia), including patients on chronic treatment for these conditions.<br>• Advanced hepatic, renal, cardiac or pulmonary disease.<br>• Terminal medical diagnoses compatible with a survival of < 1 year.<br>• Major substance dependence, including alcohol (in the investigator's opinion).<br>• Lesion of the dominant writing hand that could influence cognitive measurements or other results (in the investigator's opinion). |
| **Visit schedule** | **Visit 1 - Screening**<br>**Study Day 0**<br>• Demographic data<br>• Medical history and risk factors<br>• ICF<br>• Randomisation<br>• Primary and secondary outcomes<br>**Visit 2 - Efficacy evaluation**<br>**Study Day 90**<br>• Primary outcomes<br>• Secondary outcomes<br>**Visit 3 - Efficacy evaluation**<br>**Study Day 180**<br>• Primary outcomes<br>• Secondary outcomes |
| **Active food-supplementation arms** | Group I - N-Pep-12 (90 mg), daily (Day 0 – Day 180)<br>Group II - control |

* All treatment cycles and efficacy evaluations will be carried out within a time window of ±5 working days.

# 2. INTRODUCTION

## 2.1. Background information and rationale of the study

Traumatic brain injury (TBI) is increasingly recognised as a chronic and progressive neurological condition rather than a single acute event, with lasting effects on functional independence and quality of life (Maas et al., 2022). Recent global estimates report over 27 million new cases annually, placing TBI among the leading causes of disability worldwide and a major contributor to years lived with disability and to the socio-economic burden (Guan et al., 2023).

It is frequently described as an “invisible disability”, because many patients may present few obvious physical deficits yet continue to experience profound cognitive, behavioural and emotional dysfunction, which delays their social and occupational reintegration (Engström et al., 2025). Cognitive sequelae — such as impaired attention, slowed processing speed, memory dysfunction, and deficits of executive function and cognition — may persist for years after the trauma (Maas et al., 2022).

Neuropsychiatric manifestations are equally significant: meta-analyses show increased rates of depression and anxiety after TBI, with a prevalence of anxiety of around 17–18% and a substantially increased risk of major depressive disorder (Dehbozorgi et al., 2024a; Dehbozorgi et al., 2024b). Emotional dysregulation, impulsivity, irritability, apathy and personality changes are frequent and often more disabling than motor deficits (Howlett et al., 2022). Long-term studies also demonstrate an increased risk of suicide and a global deterioration of mental health (Ogonah et al., 2025).

All these data underline the fact that TBI remains a major public health problem, with a sustained impact on individuals, families and healthcare systems.

N-PEP-12 is a peptide-based nutritional supplement that has been shown in experimental studies and in early clinical studies to have neuroprotective and pro-cognitive effects in patients with age-related cognitive impairment (Crook, Ferris, Alvarez, Laredo & Moessler, 2005; Hutter-Paier, Reininger-Gutmann, Wronski, Doppler & Moessler, 2015).

After only 30 days of daily administration of 90 mg N-PEP-12, a significant improvement in memory impairment was observed in elderly people with subjective memory complaints (Crook et al., 2005). MemoProve and Cebrium are two commercial products containing the substance, available in the form of tablets or film-coated capsules.

Previous research has shown that the peptides in N-PEP-12 display properties similar to natural peptide-based growth factors, such as stimulation of neurite outgrowth, increased neuronal survival and protection against metabolic stress (Windisch, Hutter-Paier, Grygar, Doppler & Moessler, 2005).

The rationale for this study is based on these previously documented properties, which have the potential to influence attentional performance, cognition and mental well-being in patients with post-TBI neurocognitive impairment.

# 3. STUDY OBJECTIVES

## 3.1. Primary objective

To evaluate the efficacy of daily supplementation for 180 days with 90 mg N-Pep-12 on neurocognitive function and on the neurorecovery outcome in patients with post-TBI cognitive impairment, on day 180 of the study.

### 3.1.1. Primary variables

- Montreal Cognitive Assessment (MoCA)
- Hospital Anxiety and Depression Scale (HADS)
- Digit Span (DS)
- Color Trial Test (CTT)
- Processing Speed Index (PSI)
- Rey Auditory Verbal Learning Test (RAVLT)

## 3.2. Secondary objectives

To evaluate the safety of daily supplementation for 180 days with 90 mg N-Pep-12 on neurocognitive function and on the neurorecovery outcome in patients with post-TBI cognitive impairment, on days 30 and 90 of the study.

### 3.2.1. Secondary variables

- AE / SAE
- EQ-5D-5L

  Subgroup analysis:
- QEEG parameters
- Eye Tracking parameters

# 4. STUDY DESIGN

Randomised, placebo-controlled, single-blind

Study group 1: 90 mg N-PEP-12

Study group 2: control

The study will be conducted in a sample of patients with a clinical diagnosis of traumatic brain injury, at 30 days post-event, who present residual neurocognitive disturbances. The study extends over an observation period of 180 days. Three visits for neuropsychological evaluation are planned over the course of the study.

**Visit 1 - (Study Day 0) – Screening + Baseline**

- ✓ Initial evaluation – demographic data, medical history and the patient's risk factors will be collected; it is verified whether the patient meets the inclusion/exclusion criteria.
- ✓ Before signing the informed consent form, the purpose of the study, the procedures involved, the duration, the potential benefits and the possible risks are explained to the patient in detail. All relevant aspects are explained in terms the patient can understand, including the rights they have as a participant and the freedom to withdraw at any time, without consequences for their medical care. The patient had the opportunity to ask questions, and all uncertainties were clarified, ensuring that they fully understood the information before deciding to sign the consent.
- ✓ After the explanations, the patient signs the consent, following which they are randomised into one of the two groups (the group receiving the study medication – N-Pep-12 90 mg, or the control group).

- ✓ The neuropsychological scales are applied for the evaluation of the primary and secondary variables.
- ✓ Randomisation of patients will be carried out by the study nurse, and not by the evaluating physician, given that the study is organised in a single-blind design and the investigator must not know the treatment allocation.
- ✓ The study nurse manages the randomisation procedure according to the pre-established list and is responsible for dispensing the study supplement to the patient, providing the necessary instructions for administration. The patient receives the corresponding medication until the next evaluation visit, at which point adherence is checked and, where applicable, the next set of supplements is provided.

**Visit 2 - (Study Day 90)**

- ✓ The neuropsychological scales are applied for the evaluation of the primary and secondary variables.
- ✓ Adverse events / severe adverse events are recorded.
- ✓ The patient receives the corresponding medication until the next evaluation visit, at which point adherence is checked and, where applicable, the next set of supplements is provided.

**Visit 3 - (Study Day 180)**

- ✓ The neuropsychological scales are applied for the evaluation of the primary and secondary variables.
- ✓ Adverse events / severe adverse events are recorded.

* All treatment cycles and efficacy evaluations will be carried out within a time window of ±5 working days.

# 5. SELECTION AND WITHDRAWAL OF PATIENTS

## 5.1. Inclusion criteria

- Patients with mild, moderate or severe traumatic brain injury (TBI) established following a neurological/neurosurgical medical examination
- Patients in the post-acute stage of TBI, at least 30 days after the TBI, with a stable clinical condition
- Presence of residual cognitive and/or affective dysfunction, documented by neuropsychological assessment (MoCA and/or HADS and/or persistent subjective neurocognitive complaints)
- Isolated traumatic brain injury (AIS score ≤ 2 in other parts of the body).
- Karnofsky performance index prior to the trauma = 100.
- Age between 18 and 80 years.
- Patient able to give written informed consent for inclusion in the study.
- Patient willing and able to comply with the requirements of the protocol for the entire duration of the study.
- Women of childbearing potential must have a negative urine pregnancy test and must agree to use adequate contraceptive methods.
- The patient must have been able to speak, read and write before the accident.

## 5.2. Exclusion criteria

- Patient with polytrauma (AIS score > 2 in other parts of the body).
- Patient with spinal cord lesions.
- Major pre-existing and active psychiatric disorder (for example: major depression, schizophrenia, bipolar disorder, dementia), including patients on chronic treatment for these conditions.
- Advanced hepatic, renal, cardiac or pulmonary disease.
- Terminal medical diagnoses compatible with a survival of < 1 year.
- Major substance dependence, including alcohol (in the investigator's opinion).
- Lesion of the dominant writing hand that could influence cognitive measurements or other results (in the investigator's opinion).

## 5.3. Criteria for interruption and stopping

### 5.3.1. Study-related interruption criteria

- Insufficient recruitment
- Continuous violations and significant deviations from the protocol

### 5.3.2. Patient-related interruption criteria

Patients will be notified in the informed consent about their right to withdraw from the study at any time, without prejudice, but also that they may be withdrawn at the discretion of the Investigator / Coordinator at any time. If a patient drops out or is withdrawn from the study, the drop-out/withdrawal page of the case report form (CRF) will be completed. The Investigator must record the date of withdrawal, the person who initiated the withdrawal and the reason for withdrawal. In order to complete the evaluations and the collection of any data or other study resources, reasonable efforts should be made to contact any patient who has been lost to follow-up during the study.

*The patient was withdrawn by the Investigator because of:*

- Significant adverse effects
- Withdrawal of consent
- Other administrative reasons

*The patient or their legal representative requested withdrawal because of:*

- An adverse effect for which the Investigator did not consider withdrawal from the study necessary
- The therapeutic effect being perceived as limited
- Withdrawal of consent for any other reason (the data recorded up to the withdrawal will be kept in the database if the patient does not expressly object to this)

## 5.4. Randomisation and data management

This study will be conducted in a single-blind manner, in order to maintain confidentiality of the treatment allocation with respect to the Investigator.

Randomisation of patients will be carried out by the study nurse, and not by the evaluating physician, given that the study is organised in a single-blind design and the investigator must not know the

treatment allocation. The study nurse manages the randomisation procedure according to the pre-established list and is responsible for dispensing the study supplement to the patient, providing the necessary instructions for administration. The patient receives the corresponding medication until the next evaluation visit, at which point adherence is checked and, where applicable, the next set of supplements is provided.

Patients who meet the inclusion criteria will be allocated a unique randomisation number (patient code). This will be the closest available number in ascending order from 001 to, for example, 999, in accordance with a predefined randomisation plan; on this basis the treatment allocated to a unique patient will be identified in a single-blind manner, from a list generated in advance at random by a statistician chosen by the Coordinator. Patients will be allocated to the study groups at random and in a 1:1 ratio. Separate randomisation lists will be generated for each study centre. Randomisation will be carried out in blocks of four patients.

A list with the patient codes will be prepared and sealed, together with sets of sealed envelopes. On the basis of the list, opaque and sealed randomisation/emergency envelopes will be provided to:

- The study centre – for unblinding in cases where a reasonable suspicion of harm to the patient arises
- The nurse designated to administer the study medication
- The study Coordinator

When the randomisation/emergency envelopes are opened, they will be dated (date, time) and signed by the person opening them. Any premature unblinding of the Investigational Product must be documented and explained to the Coordinator as soon as possible. After the database is closed and the analysis populations are established, the entire study will be unblinded.

# 6. INVESTIGATIONAL PRODUCT

The Investigational Product will be made available by the Coordinator (FSNN).

## 6.1. Name and description of the Investigational Product

N-Pep-12 (Cebrium/MemoProve)

## 6.2. Doses, formulations and administration

*Active treatment - N-Pep-12: 90 mg*

*Control arm: no medication*

## 6.3. Packaging and labelling

The Investigational Product and its reference will be packaged and labelled for use in the clinical study, in accordance with GMP Annex 13 and local legislation.

## 6.4. Storage

The study medication will be kept at room temperature, in a dry place. All supplies must be kept in a safe, locked place, inaccessible to unauthorised persons, until the moment of delivery to the patients.

### 6.5. Accountability for the Investigational Product and its destruction

The quantity of medication used will be recorded in the CRF. Unused medication will be inventoried, documented and subsequently destroyed.

## 7. CONCOMITANT MEDICATION

All medicinal products used previously or concomitantly must be recorded in the CRF, including the start date, the discontinuation date (where applicable), the highest daily dose administered and the route of administration. In addition, the reason (diagnosis) underlying the administration of the medication must be documented. If a concomitant medicinal product is used prophylactically, this must be documented on the corresponding page of the CRF.

## 8. DEFINITION OF THE PRIMARY AND SECONDARY VARIABLES

### 8.1 Primary variables

#### 8.1.1 Montreal Cognitive Assessment

The Montreal Cognitive Assessment (MoCA) is a cognitive screening instrument whose administration takes approximately 10 minutes. It evaluates multiple cognitive domains, including attention, concentration, executive functions, memory, language, visuo-constructive abilities, conceptual thinking, calculations and orientation. The total score ranges between 0 and 30, with one additional point awarded to persons with ≤12 years of education (Nasreddine et al., 2005).

#### 8.1.2 Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) is a short, 14-item self-report instrument, developed to assess symptoms of anxiety and depression while minimising somatic items that may interfere in patients with medical conditions. It includes two subscales—HADS-Anxiety and HADS-Depression—each with 7 items rated on a Likert scale from 0 to 3, resulting in scores between 0 and 21. Higher scores indicate greater severity of symptoms, with thresholds frequently used of 0–7 (normal), 8–10 (borderline) and 11–21 (clinically significant). The scale demonstrates good reliability and validity in both clinical and non-clinical settings (Zigmond & Snaith, 1983).

#### 8.1.3 Digit Span

The Digit Span test evaluates verbal working memory, attention and comprehension, which also contribute to performance. The test is closely related to language learning abilities. Working memory assessment procedures are widely accepted. A list of digits is read aloud, at a speed of one digit per second, and the participant must reproduce the digits in the correct order. The first list begins with three digits and increases progressively until the person begins to make errors (two consecutive errors). This test can be administered both forwards and backwards. Scores are considered to be correlated with age, not with the level of intelligence (Wechsler, 2008).

#### 8.1.4 Color Trial Test

The Color Trails Test (CTT) is a culturally neutral neuropsychological scale, used for the evaluation of sustained attention, processing speed, sequencing and executive functioning. It was developed as an alternative to the Trail Making Test, so as not to depend on knowledge of the alphabet. During the task, participants must connect, in the correct order, numbered circles printed in alternating colours, which reduces the influence of literacy. The test consists of two parts: CTT-1, which evaluates visual

scanning and psychomotor speed, and CTT-2, which evaluates sequencing and cognitive flexibility. Longer completion times indicate poorer performance (D'Elia et al., 1996).

#### 8.1.5 Processing Speed Index

The Processing Speed Index (PSI) is a component of the WAIS scale, designed to evaluate the speed and efficiency with which a person can perceive visual information, carry out simple cognitive operations and produce motor responses. It usually includes subtests such as Symbol Search and Coding, which require rapid visual scanning, discrimination and graphomotor coordination. PSI scores reflect the individual's ability to process information rapidly under time pressure, with lower scores indicating reduced efficiency (Wechsler, 2008).

#### 8.1.6 Rey Auditory Verbal Learning Test

The Rey Auditory Verbal Learning Test (RAVLT) is an instrument widely used for the evaluation of verbal learning and memory, analysing immediate recall, rate of learning, susceptibility to interference, delayed recall and recognition. During the test, participants are presented with a list of 15 unrelated words, repeated over five learning trials, followed by an interference list and then immediate and delayed recall tasks. RAVLT provides sensitive indicators of the efficiency of encoding, retention and retrieval processes, being useful both in the clinical setting and in research, for identifying subtle memory deficits (Rey, 1964).

### 8.2.1 Secondary Variables

#### 8.2.1.1 Adverse events (AE)

#### 8.2.1.2 Severe adverse events (SAE)

#### 8.2.1.3 EQ-5D-5L

EQ-5D-5L is divided into two sections: the descriptive system and the visual analogue scale. The five dimensions of the descriptive system are: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. For each dimension there are five levels: no problems, slight, moderate, severe and extreme problems. The patient ticks the statement that best describes their current health state in each dimension. Each choice generates a digit representing the selected level. The digits of the five dimensions can be combined into a five-digit number indicating the patient's health state (Herdman et al., 2011).

### 8.2.2 Subgroup analysis

#### 8.2.2.1 Eye Tracking

Eye movements will be recorded with an infrared eye-tracking system (Tobii TX300), with a temporal resolution of 300 Hz. The target stimulus, created in the Tobii Studio program, will be presented on a screen placed 40 cm from the subject, in a darkened room. Before testing, an optotype will be used to verify that all subjects have normal or corrected-to-normal vision. A 9-point calibration will be carried out, including the centre and the periphery, before each session, in order to ensure a complete visual field and adequate eye movements.

#### 8.2.2.2 32 Channel – qEEG

Continuous qEEG recordings will be performed both at rest and during cognitive tasks, in the following sequences: 1) eyes open – 5 minutes, 2) eyes closed – 5 minutes, 3) cognitive tests – 10 minutes, 4) eyes open – 5 minutes, 5) eyes closed – 5 minutes.

Scalp electrodes fixed in an elastic cap will be used, positioned according to the international 10–10 system, and electrode impedances will be kept below 5 kΩ. The qEEG data will be recorded using 32 channels.

# 9. EVALUATION AND REPORTING OF ADVERSE EVENTS

During the clinical study, increased attention will be paid to the adverse events and adverse drug reactions mentioned below.

## 9.1. Adverse events (AE)

A serious adverse event (SAE) is an unfavourable medical occurrence appearing in a patient or subject of the clinical study who has received a pharmaceutical product, without there necessarily being a link between the event and the treatment in question. An adverse event (AE) is, consequently, any unfavourable and unforeseen sign (including an abnormal laboratory finding), symptom or disease that is temporally associated with the use of the Investigational Product, whether or not there is a link between them.

## 9.2. Adverse drug reactions (ADR)

ADR means all negative and unintended reactions to the Investigational Product caused by the administration of any dose of it. The term “response to the Investigational Product” refers to a reasonable causal link established by the Investigator or the Coordinator. “Reasonable” generally presupposes that there is an indication or argument suggesting a causal relationship. Regarding Investigational Products placed on the market: a reaction to the product that is negative, unintended and that occurs in the case of usual applications used in humans for the prevention, diagnosis or treatment of a disease, or to modify a physiological function.

### 9.2.1. Serious adverse events or serious adverse reactions (SAE/SADR)

SAEs will be taken into consideration for the documentation of AEs due to the patient’s constitution. SADRs will be addressed as set out below.

Urgent reporting is required if the following criteria apply (ICH E2A):

1. Serious
2. Unexpected
3. Reasonable causal relationship with the studied treatment

An adverse drug reaction is considered serious if it:

- Results in death
- Is life-threatening for the patient
- Requires prolongation of hospitalisation or a new hospitalisation
- Causes significant or persistent disability/incapacity
- Causes a congenital anomaly or birth defect
- Causes other significant medical events requiring immediate medical or surgical intervention

Unexpected means:

- It does not correspond to the Investigator’s Brochure or the Summary of Product Characteristics (SmPC)

Causal relationship means:

- There are facts/evidence suggesting a causal relationship
- Suspicion of a reasonable causal relationship, according to the medical staff

**NOTE**

**Death:** as a result of an Adverse Event. The medical cause of deaths, such as an accident or a pre-existing condition, must be reported in detail.

**Life-threatening:** in the definition of SAE or SADR this refers to a real event in which the patient was at risk of death; it does not refer to an event which, hypothetically, could have caused the patient's death in the case of increased severity.

In other cases, medical judgement must be applied to establish whether an AE or an ADR was serious. Important AEs/ADRs which are not immediately life-threatening and do not lead to death or prolonged hospitalisation, but which endanger the patient or require interventions to prevent the consequences mentioned above, should be considered serious.

## 9.3. Suspected expected serious adverse reaction (SUSAR – expected)

Any adverse reaction which, by its nature, is serious and which corresponds to the information available about the product in question in the SmPC.

## 9.4. Suspected unexpected serious adverse reaction (SUSAR – unexpected)

Any adverse reaction which, by its nature, is serious and which does **not** correspond to the information available about the product in question in the SmPC.

## 9.5. Recording of adverse events

All adverse events, serious or not, will be documented and, where necessary, reported in accordance with the definitions given above.

On the corresponding page of the CRF, the Investigator must detail all adverse signs and symptoms reported by patients or observed during or after the treatment cycles with the administered Investigational Product.

The nature of the signs and symptoms; the date of onset; the date of resolution (duration); the severity/intensity; the relationship with the study treatment or other treatments; the actions taken (in cases where this was necessary); and the outcome of the event should be included in the description.

### 9.5.1. Definition of the intensity of adverse events

| Intensity | Definition |
|---|---|
| Mild | The patient is aware of signs and symptoms, but these are easily tolerated |
| Moderate | The signs/symptoms cause discomfort to a degree sufficient to interfere with usual activities |
| Severe | The patient is unable to work or to carry out usual activities |

### 9.5.2. Definition of the causality of adverse events

Based on the WHO-UMC system for the standardised evaluation of causality (www.who-umc.org), the following categories will be used to describe the degree of causality (reasonable conformity with all the points is required):

**Certain**

- Event or abnormal laboratory finding, with a plausible temporal relationship to the moment of administration of the studied medicinal product
- Cannot be explained by a disease or other medicinal products
- Plausible response to withdrawal of the medicinal product (pharmacological, pathological)
- Event that is clear from a pharmacological or phenomenological point of view (i.e., an objective and specific medical condition or a recognised pharmacological phenomenon)
- Satisfactory rechallenge, if needed

**Probable**

- Event or abnormal laboratory finding, with a reasonable temporal relationship to the moment of administration of the studied medicinal product
- Unlikely to be explained by a disease or other medicinal products
- Reasonable clinical response to withdrawal of the medicinal product (for details see WHO-UMC)
- Rechallenge is not necessary

**Possible**

- Event or abnormal laboratory finding, with a reasonable temporal relationship to the moment of administration of the studied medicinal product
- Could be explained by a disease or other medicinal products
- The information regarding withdrawal of the medicinal product is insufficient or unclear

**Unlikely**

- Event or abnormal laboratory finding, with an improbable (but not impossible) temporal relationship to the moment of administration of the studied medicinal product
- A disease or other medicinal products could be plausible explanations

**Unrelated**

- The event does not follow a reasonable temporal sequence from the administration of the medicinal product and is clearly related to other factors, such as the clinical condition, therapeutic interventions or other medicinal products

**Not assessable**

- The adverse reaction is suggested by a report
- It cannot be assessed because of insufficient or contradictory information
- The data cannot be supplemented or verified

All cases assessed by one evaluator or by any two evaluators as having “a reasonable causal relationship” with the Investigational Product qualify as ADRs. This situation falls within the categories “certain”, “probable” and “possible”.

### 9.6. Reporting of serious adverse events

All SAEs and unexpected SUSARs with a reasonable suspicion of a causal relationship with the Investigational Product must be reported to the Coordinator within 24 hours (on working days) from the moment the Investigator became aware of them. SAEs will be reported preferentially by email to research@ssnn.ro.

### 9.7. Exceptions from urgent reporting

Not applicable.

### 9.8. Post AE/ADR procedure

AEs/ADRs will be followed up throughout the clinical study and any change will be recorded in the CRF.

## 10. STUDY SCHEDULE

### 10.1. Procedure during the visits

**Visit 1 - Screening - Study Day 0**

- Demographic data
- Medical history and risk factors
- ICF
- Randomisation
- Primary and secondary outcomes

**Visit 2 - Efficacy evaluation - Study Day 30**

- Primary outcomes
- Secondary outcomes

**Visit 3 - Efficacy evaluation - Study Day 90**

- Primary outcomes
- Secondary outcomes

**Visit 4 - Efficacy evaluation - Study Day 180**

- Primary outcomes
- Secondary outcomes

**Visit 5 - Telephone efficacy evaluation - Study Day 270**

- Primary outcomes – limited set
- Secondary outcomes – limited set

### 10.2. Compliance evaluation

Compliance will be documented in the CRF by recording the date and time of administration.

### 10.3. Risk evaluation and precautionary measures

The Investigational Product has been used in clinical practice for many years and has demonstrated a benign safety profile. Information regarding the safety of the Investigational Product is set out in the SmPC (Annex 1).

## 11. DURATION OF THE STUDY AND OF THE TREATMENT

*Start date of the study/treatment: 10/2022*

*End date of the study/treatment: 06/2024*

## 12. STATISTICAL METHODS

The final statistical analysis of the study will be carried out by a qualified biostatistician and will meet all the ICH-GCP requirements for clinical study data. The statistical analysis, including any subgroup analysis, will be agreed before the evaluation of the data, and the results will be established in a statistical analysis plan that will be finalised before the data are unblinded. The study data will be analysed and the statistical report will be drawn up as soon as all the data have been entered into the database and validated.

The analysis will be based on the pre-specification of the primary analyses and of the control over the multiple alpha level. The power of this study is calculated using the following parameters:

- K=2 experimental treatments will be included in the study.
- A significance level of $\alpha=0.05$ will be used, in combination with the Dunnett correction.
- The standard deviations of the response are assumed to be: $(\sigma 0,\ldots,\sigma K)=(1,1,1)$.
- The minimum marginal power will be controlled at the level $1-\beta=0.9$ under each of the corresponding least favourable configurations.
- The treatment effects will be: $\delta 1=0.5$ and $\delta 0=0$.
- The target allocation for the experimental arms will be identical to that of the control arm.
- Consequently, the allocation ratios realised in the experimental arms are: $(r1,\ldots,rK)=(1,1)$.
- The maximum family-wise error rate is: 0.05.
- The minimum marginal power is: 0.9.
- The critical threshold suggested for use together with the multiple-comparison correction is: 0.028.
- Adjustment for patients who drop out: 10%
- The total sample size is: N=270. The sample size for each arm is: $(n0,\ldots,nK)=(90,90,90)$.

*Source: Grayling MJ, Wason JMS (2020) A web application for the design of multi-arm clinical trials. BMC Cancer 20:80. DOI: 10.1186/s12885-020-6525-0*

The primary objective of the study is the evaluation of the efficacy of N-PEP-12 from the perspective of the results obtained on the Attentional Performance Test among healthy adults of middle age and above, 90 days after initiation.

*Missing data*

Missing data of the “missing completely at random” (MCAR) type will not, in principle, influence the results. The analysis procedure should be able to cope with partially missing data of this kind. In

many studies, this type of data is replaced on the basis of “Last Observation Carried Forward” (LOCF), as long as preceding measurements exist.

In such a study there is also the possibility that “missing not at random” (MNAR) data appear: study participants who have died or who were unable to undergo the tests because of cerebral deficits. Neglecting these missing data may influence the results.

In order to identify the type of missing data, the scales will be coded for each patient and visit according to the following scheme:

1 = valid (complete task)

2 = impossible to complete (reason related to the head injury) [description of reason]

3 = incomplete (other reason, unrelated to the head injury) [description of reason]

For the scales coded “2”, the lowest value will be entered for the respective patients, since these data are not missing at random (MNAR). These data are replaced by the lowest corresponding score of the respective scale.

For the scales coded “3”, a “Last Percentile Carried Forward” (LPCF) replacement will be carried out, as long as preceding evaluations exist. This method transfers the information of the patient population on the basis of the percentile reported on the raw scale, instead of the last value (as happens in LOCF). This approach was developed recently in accordance with the recommendations of O’Brien, Zhang and Bailey (2005) for the analysis of data on progressive chronic diseases such as dementia. According to simulation studies, the calculated estimates should be influenced negligibly by the missing data. The method is more or less identical to LOCF in the case where there are no general changes among the patients. If there are no preceding measurements, the scale will be kept as missing.

*Study populations and protocol violations*

Before the study is unblinded, a blinded evaluation will be carried out. In this process, possible protocol violations will be classified as “severe”, “major”, “minor” or “non-existent”. Possible protocol violations will also be recorded in the patient databases. The analysis populations (Intention-to-treat - ITT and Per-protocol - PP) will be listed individually in the final statistical analysis plan.

The ITT population is defined by all patients for whom there were no “severe” violations of the inclusion criteria, who had at least one administration of the study medication and at least one recorded post-baseline observation for at least one primary efficacy criterion (definition according to ICH E9 - 5.2. Analysis Sets). The ITT population will be used for all efficacy analyses.

A sensitivity analysis will be carried out for the PP data set, through an exploratory approach. The PP population includes all patients eligible for the ITT evaluation and who, in addition, do not record “major” deviations from the protocol. The PP analysis will be granted a scientific relevance equivalent to that of the ITT analysis, since it reflects most closely the scientific model presented by the protocol (ICH E9 – Section 5.2.2).

The homogeneity analysis at baseline will be carried out for the ITT population. In addition to the descriptive analysis (e.g., 95% confidence intervals at both ends), the comparability analysis at baseline will illustrate the demographic and anamnestic variables, as well as the primary efficacy criteria at baseline. This allows the comparison of the variables at baseline for different scales and types of data. In the case of heterogeneity, stratified analyses will be carried out. Patients with compliance to the study medication of under 80% will be marked as protocol violations and will not be taken into consideration for the PP analysis.

An evaluation of the blinded data will be carried out in accordance with the requirements of ICH E9. The statistical analysis plan will be finalised by the statistician before unblinding takes place. The

analysis populations will be listed individually in the final statistical analysis plan. The finalisation of the statistical analysis plan and the moment of unblinding of the study will be formally documented.

*Recalculation of the sample size*

Recalculation of the sample size represents an important issue in clinical studies. While studies of larger size have a greater chance of having sufficient power, exposing more patients than necessary to answer a scientific question may be unethical. Calculation of the sample size on the basis of the estimated parameter may not be precise. Therefore, a re-estimation of the sample size will be carried out during the interim analysis, in order to provide the opportunity to re-evaluate the uncertainties using the data collected and to continue the study with an updated sample size (Wu, Lin, & Chow, 2016).

The data analysis will be carried out in a validated working environment, in accordance with the requirements of ICH E3 (1995). The evaluation program used will be described in the final statistical analysis plan.

# 13. ACCESS TO DATA AND DOCUMENTS

The Investigator will permit study monitoring, audits, inspections and verifications by the Ethics Committee, providing direct access to the primary data that constitute the source of the data in the CRF (i.e., medical documents).

## 13.1. Source data

Source data are all the information in the original observation charts or those originating from other activities of the clinical study. The source documents (originals or certified copies) contain the source data.

## 13.2. Source documents

The original documents and other records are defined as source documents (e.g., hospital documents, observation charts, laboratory results, memoranda, patient diaries, evaluation lists, pharmacy records, data recorded automatically by various medical devices, certified copies or manuscripts, photographic film negatives, magnetic information storage media).

## 13.3. Direct access

Direct access is defined as the right to inspect, analyse, verify and reproduce any document or record relevant to the evaluation of the clinical study. Any party that has direct access (e.g., national or foreign authorities, the Coordinator or its authorised representatives such as Monitors and Auditors) should take all the necessary precautions to comply with the applicable regulatory requirements for the protection of the identity of the patients and of other information belonging to the Coordinator.

# 14. QUALITY CONTROL AND QUALITY ASSURANCE

## 14.1. Quality control

Quality control is defined as the set of technical operations and activities, such as monitoring, carried out in the context of the quality assurance system for the fulfilment of the quality requirements by the study activities.

In order to ensure that all the data are reliable and have been processed correctly, quality control must be applied at each stage of data processing.

### 14.2. Study monitoring

An authorised and qualified clinical study Monitor will visit the study site at regular intervals – established on the basis of the needs of the project – in order to verify adherence to the protocol and to local requirements, but also to verify the source data and to offer help to the Investigator in carrying out the study-related activities.

### 14.3. Quality assurance

Quality assurance is defined as the set of systematic and planned actions carried out to ensure the proper conduct of the study and the generation, documentation and reporting of the data in accordance with GCP and the applicable regulatory requirements.

### 14.4. Inspection

Inspection is defined as the action of a governing authority (IRB/IEC) to verify official documents, study sites, records and any other resources it considers relevant to the clinical study and which are located at the place of inspection, at the headquarters of the Coordinator's research organisation or at any other place considered relevant.

### 14.5. Audit

The audit is a systematic and independent verification of the study activities and documents, in order to determine whether the recording, analysis and reporting of the data, together with the other validated activities of the study, were carried out in accordance with the protocol, the standardised procedures, GCP and the applicable regulatory requirements. An independent audit of the study site may take place at any time during and after the study.

## 15. ETHICAL AND LEGAL CONSIDERATIONS

### 15.1. Ethical considerations

Before the start of the study, the Investigator will have received approval/a favourable opinion from the IRB/IEC for the study protocol and its amendments. The final protocol number and the date will be used to indicate the written agreement. The composition of the IRB/IEC, including the names of its members and their function in the committee (e.g., chair, specialist) must be made available for inclusion in the Trial Master File. During the study, the Investigator should provide the IRB/IEC with all the documents for verification.

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

Before the start of the study, its protocol, together with all its amendments and the CRF, will be submitted to the IEC/IRB. Before the start of any study-related procedures, IEC/IRB approval for the protocol and the amendments will be obtained.

### 15.3. Informed consent

Patients will be informed about the study procedures and about the potential risks and benefits. Before the start of any study procedures, their consent for participation in the study will be obtained.

## 15.4. Protocol modification

The Investigator or the Coordinator should not deviate from or change the protocol except in the case of a mutual agreement, prior verification and approval by the IEC for the respective amendment. The only exceptions are when the changes are necessary to avoid an immediate danger to the study participants, or when the changes refer strictly to logistical or administrative aspects (e.g., change of Monitors, change of telephone number). The party initiating the amendment must obtain written confirmation and the signature of the Coordinator and of the Principal Investigator. Amendments to the protocol will be submitted to the IEC.

## 15.5. Conduct of the study

The study will be conducted in accordance with the Declaration of Helsinki, with this protocol, with GCP (2001/20/EC, CPMP/ICH/135/95), with the designated standard procedures and with the local legislation and regulations applicable to the use of new medicinal products in the country in which the study is conducted.

## 15.6. Personal data and data protection

Data protection applies to all data obtained during this clinical study. The Investigator and the physicians involved will not disclose the names of the patients or other personal information (excluding date of birth, age and sex). The physicians must ensure that the CRF and the other documents (e.g., copies of reports) transmitted to FSNN do not contain the names of the patients. Data storage for the statistical analysis must be done only under the patient identification code. Only the Investigator and the participating physicians are authorised to allocate a personal data identification code.

If, during the study, it becomes necessary to identify the name of a person for medical reasons, all those involved are subject to the rules of confidentiality. Personal data must be stored and processed in accordance with the legislation on data protection.

## 15.7. Data processing and record keeping

### 15.7.1. Completion of the Case Report Form (CRF)

All data entered in the CRF will be identified at the beginning of the study. The Investigator must ensure that the data reported in the CRF and in the other documents are accurate, complete, legible and documented in a timely manner. For any change or correction applied to a paper CRF, the date, the signature and an explanation are required (electronic CRFs are already monitored), without covering the original data; this applies both to paper CRFs and to electronic ones. The data reported in the CRF must be in conformity with those in the source documents; any discrepancy requires an explanation. The Investigator must have the CRFs ready for the inspection carried out by the clinical Monitor within a maximum of two weeks from the completion of the recording of all the patients.

### 15.7.2. Archiving

At the end of the study, all its documents, including the previously sealed envelopes, must be transmitted to the Coordinator. These must be kept for the period established by ICH-GCP, i.e. until at least two years from the approval of the marketing authorisation application in an ICH region and until the moment when all marketing authorisation applications in an ICH region have been finalised, or until at least two years from the official discontinuation of the clinical development of the Investigational Product (CPMP/ICH/135/95), or in accordance with national regulations, whichever

period is longer, but not less than 15 years from the routine/premature completion of the clinical study.

The final report must be kept for at least two years after the withdrawal of the Investigational Product from the market. The informed consent and all the original data must be kept for at least 15 years by the director of the clinical study or by the physicians involved.

### 15.8. Confidentiality

The purpose, content and results of this study will be treated confidentially by all the persons involved.

### 15.9. Responsibilities

The responsibilities of the Investigator, of the Monitor and of the Coordinator of the clinical study in matters of data processing, storage, planning, evaluation and quality assurance are governed by the recommendations of the “International Conference on Harmonisation” (ICH) on “Good Clinical Practice” (GCP) and apply to this clinical study.

## 16. FINAL REPORT AND PUBLICATION POLICY

The Coordinator and the Investigator will agree on the final report of the study. This will be signed by the Investigator and the physicians involved.

The results of the study are intended to be published in scientific journals. The results may also be used in correspondence related to regulatory aspects. The following conditions have the purpose of protecting commercially confidential material (e.g., patents) rather than limiting publication.

All information about the Investigational Product (such as patent applications, the formula, the manufacturing process, basic scientific data, or other information provided to the Investigator by the Coordinator and which has not been previously published) is considered confidential and will remain the sole property of the Coordinator. The Investigator agrees not to use this information without the permission of the Coordinator for any purpose other than the clinical study.

The Investigator understands that the information resulting from the clinical study will be used by the Coordinator in conjunction with the development of the Investigational Product and could be disclosed to other Investigators or to regulatory authorities. In order to permit the use of the information resulting from the study, the Investigator understands that it is necessary to make available to the Coordinator all the test results and data generated during the study.

## 17. SIGNATURES

The undersigned have read this protocol and agree to conduct the study in accordance with everything stipulated by the protocol and respecting the provisions of the Declaration of Helsinki.

| | **Signature:** | **Date:** |
|---|---|---|
| **Prof. Dr. Dafin F. Mureșanu**<br>(Principal Investigator) | _____ | 15.01.2026 |
| **Nicoleta Jemna** | _____ | 15.01.2026 |

(Co-investigator)

**Olivia Verisezan-Rosu**
(Co-investigator) _____ 15.01.2026

*Note: in the original Romanian document, the signature block is signed and dated 15.01.2026 by all three signatories.*

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