CLINICAL TRIAL TITLE

Multicenter Randomized trial of Ghrelin in anterior circulation ischemic stroke treated with endovascular thrombectomy. A randomized phase 2 trial

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DOCUMENT HISTORY

Document	Date of version	Summary of Changes
Protocol version no. 1.4	*24 June 2025*	• *CTR format protocol* • *Reference to CTIS/EudraVigilance* • *More detailed description ghrelin administration*
Protocol version no. 1.3	*8 January 2024*	• *Barthel Index added as outcome measure*
Protocol version no. 1.2	*17 March 2023*	• *Primary effect variable changed*
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Original protocol	*21 October 2022*	*Not applicable*

CONFIDENTIALITY STATEMENT

This document contains confidential information that must not be disclosed to anyone other than the sponsor, the investigative team, regulatory authorities, and members of the Research Ethics Committee.

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1. ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
AxMP	Auxiliary Medicinal Product
CA	Competent Authority
CCMO	Centrale Commissie Mensgebonden Onderzoek (Central Committee on Research Involving Human Subjects)
CRF	Case Report Form
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
EU	European Union
EMA	European Medicines Agency
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
IB	Investigator's Brochure
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
MoCA	Montreal Cognitive Assessment
NIHSS	National Institutes of Health Stroke Scale
PI	Principal Investigator
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

2. SYNOPSIS

Multicenter Randomized trial of Ghrelin in anterior circulation ischemic stroke treated with endovascular thrombectomy. A randomized phase 2 trial.
EU trial number: 2024-515705-26-00

Rationale

About half of the patients with acute ischemic stroke treated with endovascular thrombectomy (EVT) remain dependent on the help of others or die in the first 90 days. We hypothesize that treatment with ghrelin, started in the first six hours after stroke onset, improves early recovery and long-term functional outcome in these patients. Ghrelin is a naturally occurring hormone and mildly excitatory neurotransmitter also known as the 'hunger hormone.' Treatment with acylated ghrelin consistently improved functional and histological recovery in in vitro and in vivo models of ischemic stroke.

Objective

The primary objective of this study is to assess the effect of ghrelin on the severity of the neurological deficit at seven days after symptom onset in patients with acute ischemic stroke caused by large vessel occlusion of the anterior circulation and treated with EVT. Secondary objectives include assessment of effects of ghrelin on functional outcome at 90 days, infarct size at 3 days, blood glucose levels and blood pressure during the first 7 days, and safety.

Main trial endpoints

The primary outcome measure is the score on the NIHSS at day 7 after stroke onset or at hospital discharge, if earlier, adjusted for the NIHSS score before EVT and other relevant baseline characteristics.

Secondary trial endpoints

Secondary outcomes include infarct size derived from MRI at day 3, blood glucose and mean blood pressure levels during the first seven days, scores on the modified Rankin Scale at 90 days, and the number of SAEs.

Trial design

Phase 2 randomized clinical trial with open-label treatment and blinded endpoint assessment (PROBE design). The intervention contrast will be intravenous acylated ghrelin in addition to standard care (intervention group) vs. standard care alone (control group). The study will be performed at the stroke units of UMC Utrecht, Rijnstate Hospital, Medisch Spectrum Twente (MST), and Isala. Subjects will be participating for 3 months.

Trial population

Patients ≥ 18 years with acute ischemic stroke caused by large vessel occlusion of the anterior circulation (distal intracranial carotid artery or middle (M1/proximal M2) cerebral artery), eligible for EVT and possibility to start the study treatment within six hours after stroke onset, with a pre-EVT score of at least 10 on the NIHSS, no pre-stroke disability (mRS ≥ 2), no intracranial haemorrhage and a life expectancy > 1 year.

Interventions

Treatment in the intervention group will consist of 600µg intravenous acylated ghrelin, twice daily for five days (12h interval). This treatment will be additional to standard treatment, including intravenous thrombolysis if indicated. Treatment in the control group will consist of standard treatment alone, without ghrelin.

Ethical considerations relating to the clinical trial including the expected benefit to the individual subject or group of patients represented by the trial subjects as well as the nature and extent of burden and risks

Treatment with ghrelin, started in the first six hours after stroke onset, may improve early recovery and long-term functional outcome in these patients.

Acylated ghrelin has been administered as an infusion or a bolus in a variety of doses to at least 1850 study participants, including patients with cardiovascular or brain diseases, and had an excellent safety profile with few adverse effects. In the recently published Ghrelin In Coma (GRECO) trial in highly vulnerable comatose patients after cardiac arrest, there were no safety issues. We assume that the risk of adverse events in patients with acute ischemic stroke is equally small.

3. INTRODUCTION AND RATIONALE

3.1 New treatments for ischemic stroke are needed

Ischemic stroke is a leading cause of long-term adult disability, worldwide. The only treatments of proven benefit are early recanalization by intravenous thrombolysis (IVT) or endovascular treatment (EVT), aspirin, and surgical decompression. About half of the patients treated with EVT in the first six hours of stroke onset remain dependent on the help of others or die in the first 90 days. There is an urgent need to uncover new treatment targets and to identify new effective treatments.[2]

3.2 Ghrelin improved brain- and functional recovery in animal models of ischemic stroke

Ghrelin is a naturally occurring 28-amino acid peptide functioning as a hormone and mildly excitatory neurotransmitter. It is mainly produced in the stomach. The bioactive form, acylated ghrelin, represents 10% of the total amount of circulating ghrelin. A primary function is signaling nutrient availability from the gastrointestinal tract to the brain. Ghrelin is present in the healthy brain, where it influences mood, sleep-wake rhythm, learning, memory, and neurogenesis.[3]

Acyl-ghrelin treatment consistently improved functional and histological recovery in animal models of ischemic stroke.[4–8] In our own lab, ghrelin treatment was associated with improved synapse recovery, reduced apoptosis, and improved functional recovery after simulated cerebral ischemia in cultured rodent[9] and human neurons.[10] Ghrelin treatment was associated with less severe damage of hippocampal CA1 neurons after transient forebrain ischemia[11] and better functional recovery, less histological neuronal damage, and less apoptosis after transient global cerebral ischemia.[12]

3.3 Mechanism of action

Ghrelin plays an important role in energy homeostasis. It is mainly known as the 'hunger hormone', since an increase in ghrelin blood levels leads to feeling hungry.[13] Acyl-ghrelin passes the blood brain barrier and acts as a mildly excitatory neurotransmitter with its own, specific receptor sites,[14,15] where it plays a regulatory role in learning and memory[16,17] with neuroprotective effects in models of neurodegenerative diseases.[18,19]

Intracellularly, acyl-ghrelin regulates energy homeostasis by balancing energy production (on the mitochondrial level) and cellular (neuronal) activity.[13,20] Proposed neuroprotective working mechanisms under conditions of cerebral hypoxia / ischemia include optimization of energy homeostasis by (amongst other) direct modulation of mitochondrial respiration, scavenging of reactive oxygen species, and preservation of synaptic functioning.[13] In addition, in models of cerebral ischemia, it had anti-inflammatory, vasodilatory, and neurogenesis properties.[3,20] A consistently reported final common path to improving outcome in animal models of cerebral ischemia and neurodegenerative disorders is reduction of apoptosis.[4–8]

3.4 Effects of ghrelin treatment in neurological or cardiovascular diseases

In patients with divergent neurodegenerative diseases, ghrelin treatment was associated with improved neurological functioning.[3] In patients with cardiovascular diseases, ghrelin treatment was associated with improved cardiac functioning.[1] In case-control-studies, ghrelin blood levels were lower in stroke patients than in controls,[21] but trials with ghrelin treatment in stroke patients are lacking.

3.5 Rationale for Dose Regimen/Dose Justification

In all preclinical and clinical studies, intravenous administration was used, either as a bolus or as continuous infusion.[1] We chose for bolus (short-term) infusion, because (1) bolus (short-term) infusion suits with the natural evolution of endogenous ghrelin concentrations, which fluctuates over time (most healthy individuals experience three ghrelin peaks over the day, before breakfast, lunch, and

dinner), and (2) in human studies, effects of ghrelin were larger with bolus infusions than with continuous infusion.[1] There was no difference in the percentage of participants experiencing relevant adverse events between bolus and continuous administration.[1] However, somnolence and fatigue occurred more often with bolus infusion. Therefore, to minimize the risk of relevant side effects, instead of bolus infusion in a narrower sense, we chose to apply short-term infusion with an infusion rate of max 10 µg/kg/30min, which indicates an infusion time of 30 minutes.

Starting point for the chosen regimen of 600µg/dose, two times daily, was optimization of the probability of treatment effect. In human studies, the highest tested and safe dose regimen is 10µg/kg/dose for single doses. The highest tested regimen for treatment during one week or more is 3µg/kg/dose, two times daily. In animal studies, intraperitoneal application of 10-200µg/kg/dose, two times daily during 3-8 weeks, was safe. Given these data, and with an elimination half-life of 27 to 31 minutes,[27] we chose for max 10µg/kg/dose, infused in at least 30 minutes. We tested ghrelin in the same dosage regime as proposed with the current application in the multicenter phase 2 Ghrelin in Coma (GRECO) trial in 160 patients with severe hypoxic-ischemic brain damage after cardiac arrest, a condition that shares many pathophysiological mechanisms with ischemic stroke.[47] There were no safety issues in GRECO.

We chose for a treatment duration of five days, since most damage to the brain, as well as much of the early recovery, takes place in the first days after stroke and at the same time, most patients are admitted on stroke units for approximately five days. We justify this treatment duration with previous human studies, where numbers of AEs in studies with treatments during 7, 10, or 14 days were equal to those in studies with shorter treatment durations.[1]

4. STRUCTURED RISK ANALYSIS

4.1 Potential issues of concern

4.1.1 Level of knowledge about mechanism of action

Ghrelin is a naturally occurring 28 amino acid peptide functioning as a hormone (stimulating segregation of growth hormone) and mildly excitatory neurotransmitter. It is mainly produced in the stomach. The bioactive form, acylated ghrelin, represents 10% of the total amount of circulating ghrelin. A primary function is signaling nutrient availability from the gastrointestinal tract to the brain. Ghrelin is present in the healthy brain, where it influences mood, sleep-wake rhythm, learning, memory, and neurogenesis.[3]

Intracellularly, acyl-ghrelin regulates energy homeostasis by balancing energy production (on the mitochondrial level) and cellular (neuronal) activity.[13,20] In addition, in models of cerebral ischemia, it had (amongst other) anti-inflammatory, vasodilatory, and neurogenesis properties.[3,20] A consistently reported final common path to improving outcome in animal models of cerebral ischemia and neurodegenerative disorders is reduction of apoptosis.[12]

4.1.2 Previous exposure of human beings

Ghrelin has been administered as an infusion or a bolus in a variety of doses to at least 1850 study participants, including healthy participants and patients with obesity, prior gastrectomy, cancer, pituitary disease, diabetes mellitus, eating disorders, cardiovascular disease and neurodegenerative diseaese.[1,3] There is strong evidence that ghrelin stimulates appetite and increases circulating GH, ACTH, cortisol, prolactin, and glucose across varied patient populations. There is weak evidence regarding the effects of ghrelin on LH, FSH, TSH, insulin, lipolysis, body composition, cardiac function, pulmonary function, the vasculature, and sleep. Adverse effects occurred in 20% of participants, with a predominance of flushing and gastric rumbles and a mild degree of severity. The few serious adverse events occurred in patients with advanced illness and were not clearly attributable to ghrelin treatment.[1] We tested ghrelin in the same dosage regime as proposed with the current application in the multicenter phase 2 Ghrelin in Coma (GRECO) trial in 160 patients with severe hypoxic-ischemic brain damage after cardiac arrest, a condition that shares many pathophysiological mechanisms with ischemic stroke.[47] There were no safety issues in GRECO.

4.1.3 Induction of the mechanism in animals and/or *ex-vivo*

Hypoxic-ischemic brain damage in vitro: Ghrelin treatment was associated with a 30-50% increase of physiological neuronal activity and formation of new synapses in human[10] or rodent[9] cultured neuronal networks exposed to hypoxia / ischemia.

In vivo rodent ischemic stroke models: Acyl ghrelin passes the blood-brain barrier after intravenous application.[33] Studies in a variety of animal models of neuronal injury have demonstrated neuroprotective effects.[34] In rat models of traumatic brain injury, ghrelin treatment increased survival and facilitated function recovery by suppressing inflammation and apoptosis.[35,36] In models of transient cerebral ischemia, ghrelin decreased infarct volumes and significantly reduced the expression of active cleavage products and apoptosis.[4–6] After global forebrain ischemia, ghrelin treatment was associated with less severe damage of hippocampal CA1 neurons.[11] In a state of the art model of coma after cardiac arrest, ghrelin treatment improved functional recovery and was associated with less apoptosis and less severe neuronal damage on histological examination.[12]

4.1.4 Selectivity of the mechanism

Although des-octanoyl (unacylated) ghrelin is found in higher serum concentrations, acylated ghrelin is the active form, responsible for effects in the brain. There are two variants of the central ghrelin receptor: GHS-R1a and GHS-R1b. The first has features of a typical G protein coupled receptor. The latter has been reported as an inactive form without signaling activity.[14] Following the discovery of ghrelin, various studies have demonstrated its importance in regulating food intake and body weight. These effects are largely driven by a high expression of GHS-R1a in the hypothalamus and pituitary. GHS-R1a is also expressed in the hippocampus, cortex, thalamus, raphe nuclei, ventral tegmental area, and substantia nigra,[15] mediating multiple physiological functions beyond those involved in metabolic activity. For example, ghrelin was able to regulate learning and memory, reward-seeking behavior, anxiety, and depression,[16,17] and had neuroprotective effects in neurodegenerative diseases.[18,19,37,38]

4.1.5 Analysis of potential effect

At the doses evaluated in the 66 published studies with adverse event reporting, ghrelin demonstrated an excellent short-term safety profile with few adverse effects.[1] Serious adverse events such as pneumonia, enteritis were extremely rare and difficult to attribute biologically to ghrelin administration. Most of the severe adverse events were derived from a single study of ghrelin vs. placebo administration in severely ill patients with pulmonary cachexia, a group that is vulnerable to developing additional medical problems.[26] Mild adverse events occurred in approximately 20% of participants receiving ghrelin. The most common effect was transient flushing, which occurred in 10% of volunteers, but resulted in discontinuation of study medication in only 3 of the 939 participants in whom adverse event collection was reported.[22–24] There was no difference in the percentage of participants experiencing flushing between bolus and infusion routes of administration. Larger ghrelin doses may increase the risk of flushing, as indicated by the higher rate of flushing in the 2 ghrelin bolus studies that employed the largest tested dose (10µg/kg). The most common gastrointestinal side effect was gastric rumbles, which occurred in 22 participants (2.3%) and was never severe enough to lead to ghrelin discontinuation. Gastrointestinal side effects and increased thirst were more common in volunteers who received continuous ghrelin infusions, perhaps due to the longer duration of exposure to ghrelin. Few participants developed neurocognitive effects including somnolence, fatigue, vertigo, or change in mood (26 subjects, 2.8%). These effects were more common in subjects who received ghrelin bolus, potentially due to the rapid ghrelin delivery.[1] In the GRECO trial in 160 patients with severe hypoxic-ischemic brain damage after cardiac arrest, ghrelin treatment was associated with better outcome than placebo at p = 0.06. There were no safety issues in GRECO.[47]

4.1.6 Pharmacokinetic considerations

Intravenously administered ghrelin is rapidly cleared. Fifteen minutes after administration of a single 1 or 5micrg/kg bolus, total ghrelin plasma concentrations rose to 1059 and 6599fmol/l, from a mean baseline level of 169. Elimination half-life was 27 to 31 minutes.[27] A 61-fold increase in circulating total ghrelin has been reported 1 minute after iv injection of 10micrg/kg with an elimination half-life of 10 minutes. Acylated ghrelin passes the blood brain barrier.[33]

4.1.7 Predictability of effect

There are no known biomarkers for effect of ghrelin on neurological or cardiovascular outcomes.

4.1.8 Interaction with other products

We know of no relevant pharmacokinetic or pharmacodynamics interactions between ghrelin and standard treatment modalities for patients after with acute ischemic stroke.

4.1.9 Managing of effects

There are no antidotes or antagonists available. Since all patients are initially admitted on stroke units, access to adequate medical support is guaranteed.

4.1.10 Study population

Our study population consists of patients with acute ischemic stroke caused by large vessel occlusion of the anterior circulation. These patients have a potentially life threatening disease and may be neurologically unstable. All patients are admitted to a stroke unit, intensive care unit, or other high-care facility. Our study population is vulnerable to cardiovascular or neurological adverse events.

Previously established cardiovascular effects of ghrelin were beneficial rather than detrimental. In healthy men, low-dose ghrelin infusion was associated with increased mean peak myocardial systolic velocity.[39] A large ghrelin iv bolus increased stroke volume and decreased systemic vascular resistance and mean arterial pressure (MAP) in healthy volunteers,[40] although a similar dose of sc ghrelin increased LVEF without change in MAP.[41] In participants with heart failure, higher and repeat dose infusions decreased MAP, pulmonary capillary wedge pressure, and systemic vascular resistance and increased cardiac index, stroke volume, and LVEF; these changes were associated with improved exercise capacity.[42,43] In these previous studies, cardiac effects of ghrelin were dose and route dependent, with greater potency from iv ghrelin compared to the subcutaneous route.

Previously established effects on the brain were generally “neuroprotective”.[34] In rat models of Alzheimer’s disease, intra-ventricular infusion of acyl-ghrelin during 8 days, prevented deterioration of memory function an suppressed the increased β-amyloid deposition.[44] In rat models of traumatic brain injury, ghrelin treatment increased survival and facilitated function recovery by suppressing inflammation and apoptosis.[35,36] In neuronal networks exposed to hypoxia, ghrelin treatment was associated with an increase of physiological neuronal activity and formation of new synapses.[9] In models of transient focal cerebral ischemia, mainly consisting of intraluminal vessel occlusion, ghrelin decreased infarct volumes and significantly reduced the expression of active cleavage products and apoptosis.[4,5,5,6] After global forebrain ischemia, ghrelin treatment was associated with less severe damage of hippocampal CA1 neurons.[11] In a state of the art model of coma after cardiac arrest, ghrelin treatment improved functional recovery and was associated with less apoptosis and less severe neuronal damage on histological examination.[12]

4.2 Overall synthesis of the direct risks for the research subjects

Treatment with ghrelin, started in the first six hours after stroke onset, may improve early recovery and long-term functional outcome in these patients.

Acylated ghrelin has been administered as an infusion or a bolus in a variety of doses to at least 1850 study participants, including patients with cardiovascular or brain diseases, and had an excel-lent safety profile with few adverse effects. In the recently published Ghrelin In Coma (GRECO) trial in highly vulnerable comatose patients after cardiac arrest, there were no safety issues.[47] We assume that the risk of adverse events in patients with acute ischemic stroke is equally small.

5. OBJECTIVES AND ENDPOINTS

Objective(s)	**Endpoint(s)**
Primary objective(s)	**Endpoint for the primary objective(s)**
To determine if treatment with ghrelin, started in the first six hours after stroke onset, improves neurological outcome at day seven in patients with ischemic stroke.	The primary outcome measure is the score on the NIHSS at seven days (±1) after stroke onset or at discharge, if earlier.
Secondary objective(s)	**Endpoint(s) for secondary objective(s), if applicable**
To determine if treatment with ghrelin, started in the first six hours after stroke onset, improves early recovery and long-term functional and cognitive outcome in patients with ischemic stroke, as well as infarct size and safety outcomes	• the score on the mRS at 90 days (±14) after stroke onset, • the score on Barthel index at 90 days (±14) after stroke onset, • mortality at 90 days (±14), • scores on the NIHSS at 24 (±6) and 72 (±12) hours after stroke onset, • score on the telephone version of the Montreal Cognitive Assessment (t-MoCA) at 90 days (±14), • infarct size at day 72 hours (±24) (based on MRI measurements), • blood glucose levels at days 1-7 (or until discharge), • blood pressure at days 1-7 (or until discharge), • body temperature at days 1-7 (or until discharge), • SAEs.

6. STUDY PLAN AND DESIGN

6.1 Trial Design

This will be a phase 2 multicenter clinical trial with random treatment allocation, open label treatment and blinded endpoint assessment (PROBE design). The intervention contrast will be intravenous acylated ghrelin in addition to standard care (intervention group) vs. standard care alone (control group). The study will run at the stroke units of UMC Utrecht, Rijnstate Hospital, Medisch Spectrum Twente (MST), and Isala.

6.2 Number of Patients

In total, 80 patients will be enrolled in this study, with an equal distribution across the intervention and control group.

6.3 Overall study duration and follow-up

The inclusion period will be 1 year. Follow-up duration will be three months.

6.4 Patient participation

The trial protocol, progress and results are discussed with our patient panel consisting of three to five ischemic stroke patients and partners twice a year.

7. STUDY POPULATION

7.1 Population

We will include 80 adult patients with acute ischemic stroke caused by large vessel occlusion of the anterior circulation, eligible for EVT. The trial treatment will be started within 6 hours after stroke onset. The study will be performed at UMC Utrecht, Rijnstate Hospital, MST, and Isala. In 2021, these intervention centers performed EVT in a total of about 600 patients with acute ischemic stroke. There are no restrictions with regard to sex or ethnic background.

7.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- a clinical diagnosis of acute ischemic stroke, caused by intracranial large vessel occlusion of the anterior circulation (distal intracranial carotid artery or middle (M1/proximal M2) cerebral artery) confirmed by neuro-imaging (CTA or MRA),
- treatment with EVT, defined as groin puncture in the angio suite,
- CT or MRI ruling out intracranial hemorrhage,
- a pre-EVT score of at least 10 on the NIHSS,
- age of 18 years or older,
- written informed consent (deferred),
- possibility to start trial treatment within 6 hours of stroke onset.

7.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- pre-stroke disability defined as mRS ≥ 2,
- life expectancy shorter than one year,
- child-bearing potential.

8. STUDY TREATMENTS

8.1 Investigational Medicinal Product (IMP)

8.1.1 Name and description of the IMP

Treatment in the intervention group will consist of intravenous acylated ghrelin, 600micrg dissolved in 50cc normal saline, by bolus (short term) infusion in 30 minutes, twice daily, for five days. This treatment will be additional to standard treatment, including intravenous thrombolysis, if indicated.

8.1.2 Status of development of the IMP

See Investigator's Brochure.

8.1.3 Description and justification of dosage and route of administration

Justification of intravenous route: In all preclinical and clinical studies, intravenous administration was used, either as a bolus or as continuous infusion.[1]

Justification of bolus infusion (which in this study is not bolus infusion in a narrower sense, but rather short-term infusion in 30 minutes): We chose for bolus (short-term) infusion, because (1) bolus (short-term) infusion suits with the natural evolution of endogenous ghrelin concentrations, which fluctuates over time (most healthy individuals experience three ghrelin peaks over the day, before breakfast, lunch, and dinner), and (2) in human studies, effects of ghrelin were larger with bolus infusions than with continuous infusion.[1] There was no difference in the percentage of participants experiencing relevant adverse events between bolus and continuous administration.[1] However, somnolence and fatigue occurred more often with bolus infusion. Therefore, to minimize the risk of relevant side effects, instead of bolus infusion in a narrower sense, we chose to apply short-term infusion with an infusion rate of max 10 µg/kg/30min, which indicates an infusion time of 30 minutes.

Justification of dosage: Starting point for the chosen regimen of 600µg/dose, two times daily, was optimization of the probability of treatment effect. In human studies, the highest tested and safe dose regimen is 10µg/kg/dose for single doses. The highest tested regimen for treatment during one week or more is 3µg/kg/dose, two times daily. In animal studies, intraperitoneal application of 10-200µg/kg/dose, two times daily during 3-8 weeks, was safe. Given these data, and with an elimination half-life of 27 to 31 minutes,[27] we chose for max 10µg/kg/dose, infused in at least 30 minutes. We tested ghrelin in the same dosage regime as proposed with the current application in the multicenter phase 2 Ghrelin in Coma (GRECO) trial in 160 patients with severe hypoxic-ischemic brain damage after cardiac arrest, a condition that shares many pathophysiological mechanisms with ischemic stroke.[47] We found no sign of harm or other safety issues.

Justification of treatment duration: We chose for a treatment duration of five days, since most damage to the brain, as well as much of the early recovery, takes place in the first days after stroke and at the same time, most patients are admitted on stroke units for approximately five days. We justify this treatment duration with previous human studies, where numbers of AEs in studies with treatments during 7, 10, or 14 days were equal to those in studies with shorter treatment durations.[1]

8.2 Comparator IMP(s)

Treatment in the control group will consist of standard treatment alone, without ghrelin. Treatment is open label and there will be no placebo.

8.3 Preparation and labelling of the study treatment(s)

Preparation and labelling of the investigational product will be done according to the GMP guidelines. Please see the Pharmacy Manual and label text.

9. OTHER TREATMENTS AND RESTRICTIONS

9.1 Concomitant therapy

9.1.1 Permitted medication(s)

Concomitant treatment in both groups is left to the discretion of the treating physicians, but generally follows national guidelines.[48]

9.1.2 Prohibited medication(s)

Not applicable.

9.2 Lifestyle restrictions

9.2.1 Contraception measures

Not applicable.

9.2.2 Other requirements

Not applicable.

10. TRACEABLILITY, STORAGE, ACCOUNTABLILITY AND COMPLIANCE

10.1 Traceability and storage of the study treatment

Please see Pharmacy Manual.

10.2 Accountability of the study treatment(s) and compliance

Please see Pharmacy Manual.

11. STUDY ASSESSMENTS AND PROCEDURES

11.1 Screening procedure

All patients included in this study will be seen on the emergency department, treated at the angio-suite, and admitted to a stroke unit according to current guidelines, as described in national guidelines and local protocols. Patients who meet the eligibility criteria can be considered for enrolment in the study.

11.2 Randomisation, blinding and treatment allocation

Randomisation will be performed by a trained investigator, which may be the treating physician (i.e. neurologist or resident neurology), by means of a web-based system that will provide the randomisation treatment arm (intervention or control group) which will also allocate a unique study number to each patient. Randomisation will be 1:1, in blocks of N=6-10, stratified by study site. The investigator will sign in, include the patient and randomize. The treating physician will be aware of the treatment allocation. For patients in the intervention group, the treating nurse or a physician will prepare the study medication and provide it to the patient. Assessment of the primary outcome (NIHSS at day 7) will be by an independent researcher, blinded to the treatment allocation.

The investigator or treating physician can decide to withdraw a subject from the study for urgent medical reasons. This is left to the discretion of the treating neurologist.

11.3 Study procedures and assessments

All patients included in this study will be seen on the emergency department, treated at the angio-suite, and admitted to a stroke unit according to current guidelines, as described in national guidelines and local protocols.

Patients who meet the eligibility criteria can be considered for enrolment in the study. Since effects of the treatment under study are assumed to be larger with earlier initiation, randomization and treatment should be initiated as soon as possible within 6 hours after stroke onset. The participating centers have ample experience with inclusion of patients with acute ischemic stroke in intervention studies.

Patients who are enrolled in the intervention group of the trial will receive 600µg intravenous acylated ghrelin at 12-hourly intervals (± 1 hour). The treating nurse or a physician will prepare the treatment and provide it to the patient. Intravenous ghrelin will be administered through infusion systems that are already in situ in all of the patients. There will be no additional punctures. The duration of the treatment is five days or until hospital discharge, if earlier. This treatment duration will be adhered to, both in patients with persistent neurological deficit and in patients with full recovery.

11.3.1Efficacy assessments

Baseline data will be obtained at admission as part of regular patient care and include demographic data, medical history, clinical data, imaging data, and use of medication.

National Institutes of Health Stroke Scale (NIHSS)
After randomization, daily neurological and physical examinations at the stroke unit will be performed as part of routine patient care. This includes neurological examination to collect NIHSS scores at days 1, 3, and 7. Neurological examination at day 7 (or at discharge, if earlier) will be done by an independent researcher. The NIHSS is a continuous scale to evaluate the severity of stroke by assessing a patient's performance. Scores range from 0 to 42, with higher scores indicating a more severe deficit.

Modified Rankin Scale (mRS)
The mRS is the preferred functional outcome measure in clinical stroke studies.[28] The mRS is an ordinal hierarchical scale that describes disabilities encountered post stroke, incorporating six categories from 0 (complete recovery) up to and including 5 (severe disability).[28] 'Death' is assigned a score of 6 . The mRS will be assessed at 7 (± 1) days or at hospital discharge, if earlier, by a member of local study team, and at 90 (± 14) days. Assessment of outcome on the mRS at 90 days will be performed by independent assessors, blinded to the allocated and actually received treatment. Their assessment will be based on standardized reports of a telephone interview by trained research personnel who are not aware of treatment allocation. Telephone assessment of the mRS with a structured interview has a good agreement with face-to-face assessment and can thus be used reliably in the setting of a clinical trial.[29]

Montreal Cognitive Assessment
The Montreal Cognitive Assessment (MoCA) has been recommended as a clinical screening instrument for vascular cognitive impairment. It is valid and reliable in the patients with stroke, subarachnoid hemorrhage and stroke-free persons with vascular risk factors.[30,31] A 5-minute telephone version serves as a brief, valid, and reliable cognitive screener.[32] Assessment of outcome on the MoCA-screener at 90 days will be performed by independent assessors, blinded to the allocated and actually received treatment. Their assessment will be based on small cognitive tests of attention, verbal learning and memory, executive functions/language and orientation, which will take approximately five minutes.

The Barthel index
The Barthel index (BI) score is used as an addition to the mRS to assess performance in more specific basal activities of daily life. This BI scale contains 10 items that describe different daily activities, i.e. feeding, continency, toilet use and walking. Maximum score on the BI is 100, indicating full independency in daily life. A score of 0 equals full dependency on all items. BI was demonstrated a proper measurement of disability in stroke patients[45] and reliable through telephone assessment[46]. The BI score will be assessed via telephone by a blinded researcher, simultaneously with the mRS and MoCA.

Vital signs
Blood pressure and heart rate will be assessed at baseline in the prehospital setting and at hospital admission. Where assessed as part of routine clinical practice during hospital admission, blood pressure, heart rate, and rectal or tympanic temperatures will be collected at 12-hourly intervals (± 4 hours) in the first 7 days after randomization. The method of thermometry will be noted in the eCRF. The assessment of vital signs will be discontinued at hospital discharge.

Where assessed as part of routine care, blood glucose levels will be collected.

At day 3 (±1), patients will be transported to the radiology department for MRI scanning. Before planning the scan, standard MRI contra-indications will be checked. In case of any MRI-contra-indications, the MRI scan will be omitted. If MRI is not performed because of medical or logistical reasons or because of refusal by the patient or his/ her representative, this will not be considered a protocol deviation.

Apart from the study treatment, MRI scanning, and assessment of the NIHSS at day 7, patients will not be subjected to additional procedures during admission.

Follow up at 90 days (±14) will be done by a telephone interview by a trained investigator, blinded to treatment allocation. The investigator will collect Barthel index, mRS and MoCA scores based on a standard interview that will take approximately twenty minutes.

Table. Overview of study procedures

	Baseline (Day 0)	**Day 1-5**	**Day 3**	**Day 0-6**	**Day 7**	**90 days**
Randomization	X					
Informed consent		X*				
Intravenous ghrelin treatment twice daily (intervention group only)		X				
Physical and neurological examination (within current care)				X		
MRI scan			X			
NIHSS					X	
Telephone interview						X

*: as soon as possible, but at least within 72 hours of randomization.

The total burden in hours for the patient is about 7 hours. This includes multiple transfusions of the study drug, MRI, and follow-up at three months.

11.3.2 Safety assessments

We will record 'Serious Adverse Events' (see section 13.1.2) weekly in the contexts of current care. Reporting and follow up is described in chapter 13.

12. STUDY DISCONTINUATION AND COMPLETION

12.1 Definition End of Trial

The end of the study is defined as the last patient's last visit.

12.2 Criteria for temporary halt and early termination of the clinical trial

Planned interim analyses will be performed by the trial DSMB after the first 10, 20, and 40 patients have been followed-up for 7 days. The interim analyses will only be directed at safety. The trial will be stopped because of any safety issue of the treatment under study, defined as a higher occurrence of SAEs or a 4-point higher score at the NIHSS at 7 days in the treatment than in the control group, both at $p<0.01$.

12.3 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. The reason for withdrawal, if available, will be recorded in the eCRF. Further treatment will be at the discretion of the treating physician. Follow-up will be carried out as planned. Data from non-consenting subjects will only be used when there is no written objection from the subject or representative.

Due to the deferred consent procedure, study medication has been administered to patients randomized to ghrelin before informed consent has been obtained. Therefore it is not ethical, for the safety of all patients in the study, to eliminate all information of patients who do not provide informed consent in case of a serious adverse event in the first 7 days, or death during the full study period (both important safety variables for the study). Eliminating these records could result in an underestimation of the true risk of the study treatment and reduce the validity of the data and could lead to major safety concerns for all patients in case patients with a poor outcome will selectively withdraw from study participation. To overcome this safety concern, we will register for non-consenting patients only the variables: study number, study treatment allocation, investigator-reported serious adverse events in the first 7 days (or until hospital discharge, if earlier), vital status at 90 days, and reason refusal (yes/no). All other information will completely be erased from the patient's study record.

Subjects will not be replaced after withdrawal for any reason.

12.4 Arrangements for subjects after their participation in the clinical trial ended

Not applicable.

13. SAFETY REPORTING

13.1 Definitions

13.1.1 Adverse events (AEs)

Adverse events are defined as any untoward medical occurrence in a subject to whom a medicinal product is administered and which does not necessarily have a causal relationship with this treatment.

13.1.2 Serious adverse events (SAEs)

Serious adverse event is any untoward medical occurrence in a patient or trial subject that at any dose:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect

13.1.3 Suspected unexpected serious adverse reactions (SUSARs)

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. The event must be serious;
2. There must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. The adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in the reference safety information (RSI).

13.2 Recording of AEs/SAEs/SUSARS

We will record 'Serious Adverse Events' (see section 13.1.2) weekly in the contexts of current care.

13.3 Reporting of AEs and SAEs

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events, except for the expected SAEs. Expected SAEs are events that are known to occur in the condition under study. Expected SAEs as well as study endpoints are excluded from expedited reporting but should be documented in the eCRF and reported to the Sponsor within 7 days of the Investigator's first awareness about the event. Expected SAEs are listed in Appendix A.

The sponsor will report unexpected SAEs through the web portal EudraVigilance to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. Expected SAEs will be recorded in an overview list (line listing) and reported through the web portal CTIS to the accredited METC with the yearly annual safety report.

13.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or

referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

13.5 Reporting of SUSARs by the sponsor to EudraVigilance

The sponsor will keep detailed records of all AEs which are reported to him/her by the investigator or investigators (CTR: Article 41(3)).

The sponsor will report electronically and without delay to EudraVigilance all relevant information about any SUSAR (CTR: Article 42).

The period for the reporting of SUSARs by the sponsor to EudraVigilance will take account of the seriousness of the reaction and will be as follows:

- In the case of fatal or life-threatening SUSARs, as soon as possible and in any event not later than **7 days** after the sponsor became aware of the reaction (CTR: Article 42(2(a))));
- In the case of non-fatal or non-life-threatening SUSARs, not later than **15 days** after the sponsor became aware of the reaction (CTR: Article 42(2(b))));
- In the case of a SUSARs which was initially considered to be non-fatal or nonlife threatening but which turns out to be fatal or life-threatening, as soon as possible and in any event not later than **7 days** after the sponsor became aware of the reaction being fatal or life-threatening (CTR: Article 42(2(c))).

Where necessary to ensure timely reporting, the sponsor may, in accordance with section 2.4 of Annex III, submit an initial incomplete report followed up by a complete report (CTR: Article 42(2)).

13.6 Annual safety report

Regarding investigational medicinal products other than placebo, the sponsor shall submit annually through CTIS to all Member States concerned a report on the safety of each investigational medicinal product used in a clinical trial (CTR: Article 43).

13.7 Unblinding procedures for safety reporting

Not applicable.

13.8 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will submit the notification through CTIS without undue delay of a temporary halt but not later than in 15 days of the date of the temporary halt. It shall include the reasons for such action and specify follow-up measures. The study will be suspended pending a further positive decision by the concerned member state (CTR: Article 38). The investigator will take care that all subjects are kept informed.

13.9 Urgent safety measures and other relevant safety reporting

Where an unexpected event is likely to seriously affect the benefit-risk balance, the sponsor and the investigator will take appropriate urgent safety measures to protect the subjects. In addition, the sponsor will notify the Member States concerned, through CTIS, of the event and the measures taken. That notification will be made without undue delay but no later than **7 days** from the date the measures have been taken (CTR: Article 54).

13.10 Data Safety Monitoring Board (DSMB)

The trial will be monitored by an independent DSMB. Details on the DSMB and rules on analysis and reporting are included in K5: DSMB charter.

The DSMB will perform interim analyses for safety after the first 10, 20, and 40 patients have been followed-up for 7 days. For that purpose, the DSMB will receive by email reports of SAEs and SUSARs per center. The DSMB will review numbers of SAEs and SUSARs in the intervention and control group may recommend to stop recruitment in the trial in case of more SAEs in the intervention than in the control group at $p < 0.01$, or any other safety concerns.

The advice(s) of the DSMB will only be sent to the project leaders of the study. Should the project leaders decide not to fully implement the advice of the DSMB, the sponsor will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

14. STATISTICAL ANALYSIS

14.1 Sample size, trail power and level of significance used

Sample size calculation was done in consultation with prof. dr. H. Lingsma, clinical epidemiologist at Erasmus MC. Assuming a difference between the treatment groups of 5 points on the NIHSS, an SD of 10 points, and a 20% gain in power by covariate adjustment, a sample of 80 patients would provide a power of 70% to detect a difference between the treatment groups at a significance level of 5% (two-sided tests). We consider this power of 70% to be sufficient for this phase two trial, which is intended to provide base for a sample size calculation for a subsequent randomized phase 3 trial. [25]

14.2 Primary study parameter(s)

Before follow-up will have been completed, a statistical analysis plan (SAP) will be developed that will specify: (i) Hypotheses to be tested; (ii) Treatment effects to be estimated in order to satisfy the primary and secondary objectives of the trial; (iii) Technical description of the statistical methodology and procedures for performing the statistical analysis of outcome measures and AE data; (iv) Primary, secondary, and sensitivity analyses; and (v) Subgroup analyses. The primary analysis will be a single comparison between the treatment groups with regard to of the primary outcome measure, i.e. the NIHSS score at 7 days (or at discharge, if earlier), adjusted for pre-EVT NIHSS, age, time from symptom onset to EVT, and pre-stroke score on the mRS. The primary effect variable will be the adjusted beta for the difference in the score on the NIHSS between the treatment groups. This beta will be estimated with multi-variable linear regression analysis according to the intention-to-treat principle. In additional per-protocol analyses, all patients that actually received ghrelin will be compared with patients that did not receive ghrelin. In all analyses, statistical uncertainty will be quantified by means of 95% confidence intervals.

14.3 Secondary study parameter(s)

For secondary outcome parameters, we will use simple 2x2 tables, two-group t-tests, Mann-Whitney tests, or multivariable linear and logistic regression models, as appropriate. In all analyses, statistical uncertainty will be quantified by means of 95% confidence intervals. Subgroup analyses will be based on sex, age (dichotomized at the median); and baseline NIHSS (dichotomized at the median).

14.4 Other study parameters

Baseline characteristics and raw distributions on the NIHSS will be presented in a descriptive way. Between-group differences are analyzed by means of independent samples t-tests, Mann–Whitney tests, Chi-Squared tests, or Fisher exact tests, as appropriate.
Treatment-effect modification will be explored in pre-specified subgroups defined by sex, age (dichotomized at the median), and baseline NIHSS. The statistical significance of possible differences between subgroups with regard to treatment effect will be tested with interaction terms, if relevant. No adjustments for multiple tests will be made.
All analyses will be performed with MATLAB, R, or SPSS.

14.5 Interim analysis

The DSMB will perform interim analyses for safety after the first 10, 20, and 40 patients have been follow-up for 7 days, as explained in the DSMB charter and under13.10. There will be no interim analyses for efficacy, since we consider the probability of finding convincing evidence of efficacy or futility negligible with interim analyses from this small phase 2 trial.

15. ETHICAL CONSIDERATIONS

15.1 Declaration of Helsinki

The study will be conducted according to the principles of the Declaration of Helsinki (7th revision, Fortaleza, 2013) and in accordance with the Dutch Medical Research Involving Human Subjects Act (WMO) and local guidelines. Data will be collected and processed in accordance with the General Data Protection Regulation (EU) 2016/679.

15.2 Recruitment and informed consent procedures

Patients will be recruited at emergency departments, angio suites, or stroke units of the participating hospitals. The treating physician (i.e. neurologist or resident in neurology) will check the eligibility criteria, inform the patient and (in case of incapacity) his / her legal representative shortly about the study. Subsequently, the investigator will (again) explain the study to the patient or his / her legal representative, answer questions and ask for informed consent. The investigator may be the treating physician in exceptional circumstances. Since effects of the treatment under study are assumed to be larger with earlier initiation, treatment should be initiated within 6h after stroke onset, and as soon as possible after stroke onset. The participating centers have ample experience with inclusion of patients with acute ischemic stroke in intervention studies.

This study evaluates the effect of an acute treatment in an emergency situation concerning a life-threatening disorder. In the acute stage, the very large majority of the patients will lack the capacity to decide on participation in this trial. The large majority of the patients' legal representatives will also lack this capacity because of the emergency situation, the necessity for urgent treatment and the emotional stress of the situation. Conversely, participation in the trial may be of direct benefit to the patient.
The vulnerable patient group and the importance of early treatment provide ethically and legally valid reasons for an emergency procedure where obtaining consent after the study procedure takes place (deferred consent). The trial cannot practically and ethically be carried out without deferred consent, nor can the trial be investigated in any other patient group than the one mentioned above.

A deferred consent procedure is also reasonable because of the proven safety of the IMP (see chapter4), the lack of invasive study procedures, and the likely time-dependent benefit of ghrelin. The deferred consent procedure in this trial is in accordance with the relevant Article 35 of Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use.

As soon as possible but deemed reasonable and appropriate by the investigator, but at least within 72 hours of randomization and at least before the MRI on day 3, informed consent will be obtained in accordance with the Declaration of Helsinki and ICH-GCP. The treating physician will judge the patients' capacity for providing informed consent every day during admission. The Sponsor will prepare the informed consent form (ICF) and provide the documents to the CA and REC for approval.
Before informed consent can be provided, the investigator or an authorised member of the investigational staff must explain to potential study subjects or their legal representatives the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. Subjects or their legal representatives will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the subject will receive for the treatment of his or her disease. Finally, they will be told that the local and central investigators will maintain a subject identification register for the purposes of long-term follow-up and that their

medical records may be accessed by health authorities and authorised sponsor staff, to the extent permitted by the applicable law(s) or regulation.
By signing the ICF the subject or the legal representative is authorising such access, and agrees to allow the investigators to re-contact him or her for follow-up assessments.

If the subject is considered mentally competent to provide consent, the subject will be informed and asked for consent him- or herself. However, if the subject lacks decision-making capacity, the investigator will search for a legal representative available. If there is no legal representative available, study procedures will be continued until a proxy is present.

The subject or his/her legal representative will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation, consent should be appropriately recorded by means of the subject's or the legal representative's personally dated signature and authorised study staff's personally dated signature. After obtaining the consent, a copy of the ICF must be given to the subject.

Copies or a second original of the signed ICF will be given to the subject and the original will be maintained with the subject's records. If new safety information results in significant changes in the risk/benefit assessment, the ICF should be reviewed and updated if necessary. All subjects for whom this is relevant should be informed of the new information and give their consent to continue the study.

If a patient who has been randomized refuses to participate and sign the ICF, or if his/her legal representative refuses this in case of the patient's incapacity, participation into the study will be terminated immediately. Data from non-consenting subjects will only be used when there is no written objection from the subject or representative. In an effort to describe the non-consenting population we will ask the subject or his/her representative to allow the use of routinely collected data in a coded manner. The refusal and the reason, if available, will be recorded in the patient's clinical chart.

If a patient has died before deferred consent has been obtained, his/her representative will be informed about the study treatment the patient may have received, trial procedures and use of the collected data. No consent from the representatives will be required for use of these data. A separate information letter will be given to the representative of the patient.

The original signed forms will be kept in the Investigator Site File. The PI will retain a copy of the signed informed consent forms in each patient's study record, and provide a copy to the patient or his/her legal representative.

15.2.1Objection by minors or incapacitated subjects

A vital criterion for valid consent by the patient for inclusion in a clinical trial is the patient's decision-making capacity. The criteria for assessing decision making capacity vary from country to country, but generally include four interrelated capacities: to understand relevant information, to appreciate the current situation and consequences of decisions, to use sufficient reasoning to make decisions, and to communicate a choice. We will include patients with severe stroke (NIHSS ≥ 10). The large majority of patients with severe stroke have a diminished decision-making capacity because of a reduced level of consciousness, aphasia, or another cognitive disorder. Patients with an NIHSS ≥ 10 but with a maintained capacity to provide informed consent will have considerably smaller infarcts and will have a stroke severity at the less severe end of the spectrum. Results of trials obtained in patients with the capacity to provide consent can therefore not be extrapolated to patients who cannot give consent. For these reasons, the investigators feel that it is ethically appropriate to include incapacitated patients in this trial if the informed consent of their legally designated representative has been obtained.

Incapacitated subjects will be provided study information in a way that is adequate in view of their capacity to understand it. The explicit wish of an incapacitated subject who is capable of forming an opinion to refuse participation in, or to withdraw from, the study at any time, will be respected by the investigator.
This policy is in line with 'regulation No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use, because data of comparable validity cannot be obtained in clinical trials on persons able to give informed consent, or by other research methods; MR GENTLE relates directly to the medical condition from which the patient suffers; and there are scientific grounds for expecting that participation in the clinical trial may produce a direct benefit to the incapacitated subject outweighing the risks and burdens involved if the patient will receive active trial treatment.
Incapacitated patients will be provided with trial information and will be asked to give informed consent as soon as they will have regained their decision-making capacity.

15.3 Benefits and risks assessment, group relatedness

Ischemic stroke is a leading cause of long-term adult disability, worldwide. The only treatment of proven benefit are acute recanalization by intravenous thrombolysis (IVT) or endovascular treatment (EVT) or prevention for recurrent stroke with aspirin. Only approximately 30% of patients is eligible for acute recanalization treatments and even when treated, many experience persistent functional impairments. The majority of patients is eligible for aspirin, but the number needed to treat to prevent one poor outcome is 79. There is an urgent need to uncover new treatment targets and identify new effective treatments to improve stroke outcome.[2]

Ghrelin is a naturally occurring 28 amino acid peptide functioning as a hormone (stimulating segregation of growth hormone) and mildly excitatory neurotransmitter. A primary function is signaling nutrient availability from the gastrointestinal tract to the brain. Ghrelin is present in the healthy brain, where it influences mood, sleep-wake rhythm, learning, memory, and neurogenesis.[3] Ghrelin has been tested in over one hundred human studies, including healthy volunteers, patients with (severe) cardiopulmonary diseases, and neurodegenerative diseases. Serious adverse events were rare and difficult to attribute to ghrelin.[1]

Acyl-ghrelin treatment consistently improved functional and histological recovery in *in vivo* animal models of ischemic stroke, through reduction of apoptosis.[4–8] In our own lab, ghrelin treatment was associated with improved synapse recovery, reduced apoptosis, and improved functional recovery after simulated cerebral ischemia in cultured rodent[9] and human neurons.[10] Ghrelin treatment was associated with less severe damage of hippocampal CA1 neurons after transient forebrain ischemia[11] and better functional recovery, less histological neuronal damage, and less apoptosis after transient global cerebral ischemia.[12]

We propose to measure effects of acyl-ghrelin treatment on early recovery of patients with acute ischemic stroke treated with EVT based on the significant probability of persistent functional impairments this patient group, lack of effective neuroprotective treatments to promote brain recovery, consistent beneficial effects of ghrelin under experimental *in vitro* and *in vivo* conditions, and substantial evidence of safety.

15.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

15.5 Compensation for subjects

No incentives will be given to subjects for participation in this study. Reimbursement for travel to the investigational site and/or additional costs (including food and drink) incurred by the subject caused directly by the study or its procedures may be reimbursed at reasonable and fair terms.

16. ADMINISTRATIVE ASPECTS, MONITORING AND CONFIDENTIALITY

16.1 Approval initial application and substantial modifications

The trial protocol, informed consent form, subject information leaflet, investigational medicinal product dossier, investigators brochure and any other documents required by the Regulation will be submitted for the regulatory approval before the clinical trial is started via CTIS.

The sponsor will also submit and obtain approval for substantial modifications to the original approved documents via CTIS.

A 'substantial modification' is defined in the CTR as any change to any aspect of the clinical trial which is made after notification of a decision referred to in Articles 8, 14, 19, 20 or 23 and which is likely to have a substantial impact on the safety or rights of the subjects or on the reliability and robustness of the data generated in the clinical trial.

16.2 Monitoring and Quality Assurance

A site initiation visit will be performed after it has been verified that the site is prepared for the study and that the site requirements for study participation are met. During this visit, the study personnel will get all the information needed about the protocol, study procedures and the online database.

In accordance with Good Clinical Practice (GCP) guidelines, there will be a monitor system. Herewith it will be verified that

(a) the rights and well-being of the included patients are protected
(b) reported trial data are accurate, complete, and verifiable from source documents.
(c) the conduct of the trial is in compliance with the currently approved protocol, with GCP, and with applicable regulatory requirements.

Monitoring will be done by trained personnel and with adequate frequency to ensure that the investigator's obligations are being fulfilled. Frequency and timing of monitoring visits shall be determined by the Principle Investigator for each site based on enrolment rate and volume, study compliance and findings from previous visits. Details are described in the monitoring plan.

It will be verified whether signed and dated informed consent forms have been obtained from each subject before any study related procedures are undertaken. Also, compliance with the study protocol will be checked.

16.3 Recording, handling and storage of information

For data collection, management and randomization the a web-based system will be used, which is validated and has an audit trial. The web-based system will allocate a unique study number to each patient. The principal investigator or trained and delegated member of the research team, monitor and Dutch Healthcare Inspectorate will have access to the coded source data, if necessary.

Variables that will be collected for study purposes are described in paragraph11.3. The data required for the trial will be entered by the investigation sites into eCRFs. All site staff will be trained on correct eCRF completion. Only trained personnel will receive access and be able to enter data in the eCRF. The eCRF will not be considered as source data.

Study related correspondence, patient records, signed informed consent forms and source documents are to be maintained by the study site for a minimum of 25 years after the end of this trial.

16.4 Reporting of serious breaches

The sponsor will notify the Member States concerned about a serious breach of the Regulation or of the version of the protocol applicable at the time of the breach through CTIS without undue delay but not later than **seven days** of becoming aware of that breach (CTR: Article 52).

16.5 Notification of the start and the end of the recruitment

The sponsor will notify within 15 days each Member State concerned of the start of a clinical trial in relation to that Member State through CTIS (CTR: Article 36(1)).

The sponsor will notify within 15 days each Member State concerned of the end of the recruitment of subjects for a clinical trial in that Member State through the EU (CTR: Article 36(3)).

16.6 Temporary halt/(early) termination

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last follow-up.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

16.7 Summary of the results

Within one year from the end of a clinical trial in all Member States concerned, the sponsor will submit to the EU database CTIS a summary of the results of the clinical trial. The content of the summary of the results is set out in CTR Annex IV. It shall be accompanied by a summary written in a manner that is understandable to laypersons. The content of the summary is set out in CTR Annex V (CTR: Article 37(4)).

16.8 Public disclosure and publication policy

This trial will be publicly registered in accordance with the Declaration of Helsinki on clinical trials.gov.

Publications will be by the executive committee, in the name of the steering committee. Pre-defined sub-studies or post-hoc analysis by participating investigators are possible after consultation of the executive committee and only after publication of the primary results of the trial.

The executive committee consists of H.B. van der Worp, J. Hofmeijer, and the study coordinator (PhD student). The steering committee consists of one or two additional local investigators from each participating center, and the executive committee. The steering committee will make decisions regarding continuation of the trial and protocol changes. Decisions will be prepared by the executive committee. The chairman of the steering committee (i.e. the project leader) will be advised by the independent data monitoring and safety committee. The study coordinator is responsible for running the trial on a day-to-day basis, and will report to the executive committee.

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Appendix A: Expected SAE's

Potential Serious Adverse Events after stroke:

Cardiac
Angina
Arrhythmia
Atrial fibrillation
Angina pectoris
Bradycardia
Cardiac arrest
Cardiomyopathy
Heart failure
Myocardial infarction
Tachycardia

Central nervous system
Brain oedema
Cerebral herniation
Cerebellar herniation
Delirium
Depression
Epileptic seizure
Haemorrhagic transformation of the infarct
Headache
Haematoma expansion
Hydrocephalus
Intraventricular extension of haemorrhage
Increased intracranial pressure
Progressive stroke / Stroke in evolution
Recurrent ischaemic stroke
Recurrent intracerebral haemorrhage
Recurrent stroke
Retinal ischaemia
Sleep disorder
Status epilepticus
Transient ischaemic attack
Transient monocular blindness

Gastro-intestinal
Constipation
Dysphagia
Faecal incontinence
Gastro-intestinal haemorrhage
Ileus
Melena
Mucosal irritation

Nausea
Rectal haemorrhage
Stress ulcer
Vomiting

General/other
Anaemia
Arterial hypertension
Arterial hypotension
Deep vein thrombosis
Dehydration
Fall (and consequences)
Fatigue
Fever
Haematuria
Hip fracture
Hyperglycaemia
Infections
Pain
Pressure sore
Renal failure
Sepsis
Syncope
Undernutrition
Urinary incontinence
Urinary tract infection
Extracranial haemorrhage

Pulmonary
Aspiration
Bronchitis
Central periodic breathing
Chronic obstructive pulmonary disease
Dyspnoea
Obstructive sleep apnoea
Oxygen desaturation
Pneumonia
Pulmonary embolism
Pulmonary oedema
Respiratory failure / arrest
Respiratory tract infection