CompLement C5 Antibodies for decreasing brain injury after aneurysmal Subarachnoid Hemorrhage (CLASH): study protocol for a randomized controlled phase II clinical trial

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Study protocol

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Abstract

Background

The inflammatory response after aneurysmal subarachnoid hemorrhage (aSAH) has been associated with early brain injury, delayed cerebral ischemia, poor functional outcome, and case-fatality. In experimental SAH studies, complement C5 antibodies administered shortly after SAH reduce brain injury with approximately 40%. Complement component C5 may be a new therapeutic target to reduce brain injury and hereby improve outcome after aSAH. We aim to investigate the pharmacodynamic efficacy and safety of eculizumab (complement C5 antibody) in patients with aSAH.

Methods

A randomized, controlled, open-label, phase II clinical trial with blinded outcome assessment. Eculizumab (1200 mg) is administered intravenously <12 hours, on day 3 and on day 7 after ictus. Patients in the intervention group receive prophylactic antibiotics for 4 weeks, and those with a central line or an external ventricular shunt and a positive fungal or yeast culture also receive prophylactic antifungal therapy for 4 weeks. The primary outcome is C5a concentration in cerebrospinal fluid (CSF) on day 3 after ictus. Secondary outcomes include the occurrence of adverse events, inflammatory parameters in blood and CSF, cerebral infarction on magnetic resonance imaging, and clinical- and cognitive outcomes. We aim to evaluate 26 patients with CSF assessments, 13 in the intervention group and 13 in the control group. To compensate for early case fatality and inability to obtain CSF, we will include 20 patients per group.

Discussion

The CLASH trial is the first trial to investigate the pharmacodynamic efficacy and safety of eculizumab in the early phase after aSAH.

Trial registration

Netherlands Trial Register: NTR6752, https://www.trialregister.nl/trial/6579. Registered on 27 October 2017. European Clinical Trials Database: EudraCT 2017-004307-51.

Background

Early brain injury and delayed cerebral ischemia are important determinants of poor outcome after aneurysmal subarachnoid hemorrhage (aSAH).[1] No treatment exists to reduce early brain injury and the effects of current strategies to prevent delayed cerebral ischemia are only modest.[2] The inflammatory response is considered to play a key role in the pathogenesis of early brain injury and delayed cerebral ischemia after aSAH.[3–6] Previous studies found that the complement cascade is activated in patients with SAH and associated with poor functional outcome.[7,8] Complement components C3a and C5a are proinflammatory anaphylatoxins that can induce vasoconstriction and activate coagulation,[9–11] processes that have been implicated in the pathophysiology of early brain injury and delayed cerebral

ischemia.[1,12] C5 specific antibodies, which prevent the formation of C5a, have been shown to reduce microglia activation and cell death by 40% in an SAH mouse model.[13] C5 antibodies (eculizumab) are already used for other inflammatory diseases such as neuromyelitis optica and Anti-AChR Antibody-Positive generalized Anti-AChR Antibody-Positive generalized myasthenia gravis.[14,15] The aim of this trial is to investigate the pharmacodynamic efficacy (proof-of-concept) and safety of eculizumab in patients with aSAH.

Methods

Study design

The CLASH trial is a randomized, controlled, open-label, phase II clinical trial with blinded outcome assessment (PROBE) to assess pharmacodynamic efficacy and safety of eculizumab in patients with aSAH.

Patient population and setting

Patients 18 years or older with a confirmed aSAH admitted <12 hours after ictus to the University Medical Center Utrecht (UMC Utrecht), a tertiary referral center.

Inclusion and exclusion criteria

The inclusion and exclusion criteria are presented in Table I.

Randomization

A block randomization is used to randomize patients. Patients are randomized by the investigators via a centralized secured website after informed consent from the patient or legally authorized representative is obtained. Patients are allocated 1:1 to either: 1) the intervention arm; or 2) care as usual.

Intervention

The intervention consists of intravenous infusion with eculizumab 1200 mg at three different time points: <12 hours, on day 3, and day 7 after ictus (Figure 1). The day of ictus is defined as day 1. We decided on repeated drug administration with a high dose of eculizumab to prevent a wash-out effect and because the C5a concentration in the cerebrospinal fluid (CSF) of aSAH patients is >1400 times increased one day after ictus compared to the C5a concentration in CSF from patients with unruptured intracranial aneurysms.[13] To decrease the risk of (meningococcal) infection due to eculizumab treatment, patients in the intervention group receive prophylactic treatment with ciprofloxacin during the first 4 weeks after ictus. During the recruitment phase, after inclusion of the 6th patient, we changed our protocol based on a serious adverse event (SAE) that occurred (cerebral fungal infection in patient with external ventricular shunt). After the amendment, patients in the intervention group with a central line or an external ventricular shunt and a positive fungal or yeast culture receive prophylactic ciprofloxacin and fluconazole

for the first 4 weeks after ictus. Throat and rectal swaps are performed weekly in the intervention group during in-hospital stay to test for fungus or yeast carriership/colonization and (multi-) drug resistance. In consultation with the microbiologist and infectious disease specialist, prophylactic treatment will be switched if: 1) swaps are positive for micro-organisms that require treatment and are not covered by our prophylactic regimen and; 2) resistant microbial phenotypes are found.

Data collection and management

Data collection is the same for the intervention- and control group (Figure 2). Each patient is assigned a code upon randomization. The decryption key will be stored securely and is only accessible by the investigators. All coded data will be collected in an Electronic Case Report Form (E-CRF). Samples are de-identified and stored under UNI EN ISO 9001: 2015 regulations.

Study outcomes

The primary outcome is C5a concentration in CSF on day 3 after ictus. CSF is obtained by either lumbar puncture or sampling from an external lumbar- or ventricular drain. The use of coded samples will allow blinded measurement of C5a concentration by the laboratory technician. The CSF and blood sampling, processing, storage and immunoassays are described in Additional File 2. Secondary outcomes are listed in Table 2.

Discontinuation criteria

Treatment with eculizumab will be discontinued in the following events:

- Anaphylactic shock after infusion of eculizumab. In case of a mild allergic reaction the infusion rate can be slowed down.
- Patients with Neisseria meningitis, CSF culture proven.
- Patients with meningococcal sepsis, blood culture proven.
- Other medical reasons for which the treating physician or investigator deem it necessary to discontinue treatment.

If treatment with eculizumab is halted, other study procedures will continue according to study protocol.

Sample size estimates

The sample size calculation is based on a previous study with eculizumab in patients with neuromyelitis optica.[14] In that study, C5 concentration in CSF was measured in 11 patients before and after treatment with eculizumab. In six patients, C5 was undetectable after treatment was started and in the remaining

five patients the mean C5 concentration in CSF decreased with 58%. For the CLASH trial, we conservatively assumed an overall reduction of C5 concentration in CSF with 55% and extrapolated this to a similar reduction in C5a concentration in CSF. Based on α=0.05 and β=0.20 and a standard deviation of 50%, 13 patients are needed in each group. The group size will be increased to 20 patients per group, taking into account an assumed mortality rate of 25% and 2 patients per group who refuse a lumbar puncture in a later phase despite giving informed consent earlier.

Statistical analysis

Primary analysis will be based on the per-protocol principle in which patients with CSF assessments will be included. Patients in the intervention group with CSF assessments are included if they received the first eculizumab infusion.

Primary analysis
Groups will be compared with an independent t-test or Mann-Whitney U-test, dependent on the distribution of data. If the proportion of patients categorized according to the Prognosis on Admission of Aneurysmal Subarachnoid Hemorrhage (PAASH) scale and the median Hijdra score differ between the intervention and control group, a multivariable logistic regression analysis with adjustment for these variables will be performed.

Additional analyses
Inflammatory parameters in the blood and daily Glasgow Coma Score (GSC) will be analyzed with a linear mixed model. CSF inflammatory parameters in both groups will be compared by means of an independent t-test or Mann-Whitney U-test, dependent on the distribution of the data. To compare the NIHSS score, cognition, and quality of life, a Chi-square or Fisher's exact test will be applied. A proportional odds model will be used to assess the effect of eculizumab on World Federation of Neurosurgical Societies (WFNS) score and modified Ranking Scale (mRS) score.[16]

Data monitoring body

An independent data safety monitoring board (DSMB) will oversee safety and overall conduct of the CLASH trial. Safety will be examined by ongoing monitoring of SAEs, and Suspected Unexpected Serious Adverse Reaction (SUSARs), and by an interim analysis based on SAE reporting, outcome, or case-fatality after inclusion of 20 patients. Listings of infections will be reported every two months to the DSMB chairman. The DSMB can advise early termination of the trial if there is evidence of severe harm based on SAE reporting, outcome, or case-fatality. In addition, a contract research organization will audit trial conduct following the approved monitor plan.

Discussion

In aSAH patients, an inflammatory response occurs in the subarachnoid space shortly after the bleeding. This inflammatory response has been associated with early brain injury, delayed cerebral ischemia, poor

functional outcome, and case-fatality.[1,3–5] In experimental SAH studies, treatment with complement C5 antibodies shortly after SAH reduced brain injury with approximately 40%.[13] Complement component C5 may be an important target to reduce brain injury and hereby improve outcome after aSAH. The CLASH trial is the first phase II trial to investigate the pharmacodynamic efficacy and safety of eculizumab in aSAH patients.

We chose an open-label design with blinded outcome assessment for several reasons: 1) we deemed it unethical to subject patients in the control group to 4 weeks of prophylactic treatment with ciprofloxacin and fluconazole; 2) it was not possible to manufacture placebo ciprofloxacin infusions at our hospital within a reasonable time window; and 3) this trial is a proof-of concept trial with a primary outcome based on laboratory parameters. We do not expect that ciprofloxacin or fluconazole will influence the C5a concentration in the CSF.

The CLASH trial is designed as a proof-of-concept trial and is not powered to assess effectiveness of treatment with eculizumab. Safety is an important outcome of this trial. Eculizumab treatment increases the risk of infection. Central lines or external drains can provide a point of entry for microbes. aSAH patients will therefore be closely monitored during in-hospital stay and receive prophylactic antibiotics, antifungal therapy if necessary, and a patient safety card with instructions. If our trial demonstrates efficacy and safety of eculizumab in aSAH patients, the next step will be to plan a phase III trial.

Trial Status

8 July 2019 – version 11.0

The first patient is recruited in October 2018 and currently 18 patients have been enrolled. The recruitment is anticipated to be completed by 1 April 2021, with a follow-up period until 1 July 2021. Protocol modifications will be communicated to relevant parties. The results of this trial will be published in a peer-reviewed scientific journal.

Abbreviations

aSAH: Aneurysmal subarachnoid hemorrhage

CLASH: CompLement C5 Antibodies for decreasing brain injury after aneurysmal Subarachnoid Hemorrhage

CSF: Cerebrospinal fluid

DSMB: Data safety monitoring board

E-CRF: Electronic Case Report Form

GCS: Glasgow Coma Score

mRS: Modified Ranking Scale

PAASH: Prognosis on Admission of Aneurysmal Subarachnoid Hemorrhage

PROBE: Prospective randomized open blinded end-point

SAE: Serious adverse event
SUSAR: Suspected Unexpected Serious Adverse Reaction

UMC Utrecht: University Medical Center Utrecht

WFNS: World Federation of Neurosurgical Societies

ZonMw: Netherlands Organization for Health Research and Development

Declarations

Ethics approval and consent to participate

The ethics committee of the UMC Utrecht approved this study (reference number: 17-933). Written informed consent will be obtained from the patient or legally authorized representative by the investigators or physician on call. Patients who are enrolled into the study are covered by the clinical trial insurance of the UMC Utrecht. The study will be conducted in accordance with the Good Clinical Practice E6(R2) guidelines and the current version of the Declaration of Helsinki.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

Eculizumab and research funding is provided by Alexion Pharmaceuticals under the Global Investigator-Sponsored Research Program.

Funding

The study is funded by the Netherlands Organization for Health Research and Development (ZonMw) and the Dutch Brain Foundation (PTO grant 95105015), and Alexion Pharmaceuticals (ISR grant 100237). ZonMw and the Dutch Brain Foundation are not involved in trial design, data collection, analysis, interpretation. The study drug is supplied by Alexion Pharmaceuticals, in addition to a small Investigator

Sponsored Research grant. The dosage regimen for this study was decided in consultation with Alexion Pharmaceuticals. Alexion Pharmaceuticals is not involved in any other aspects of trial design, data collection, analysis and interpretation. All authors had final responsibility for any of the above activities.

Authors' contributions

IK developed the research protocol, is responsible for conducting the study, and drafted the manuscript. GJER contributed to the study's design and revised the manuscript. MDIV conceptualized the study, is responsible for conducting the study and provided input for the research protocol and manuscript. All authors read and approved the final manuscript.

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Supplemental Material

Additional File 1: CLASH study group

Additional File 2: Sampling, processing, storage, and immunoassays

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Tables

Table 1. Inclusion and exclusion criteria

Inclusion criteria

- SAH confirmed by CT and aneurysm by CTA or DSA
- Admission to the UMC Utrecht <12 hours after ictus
- Age 18 years and older

Exclusion criteria

- Life expectancy < 10 days
- Pregnant or breast-feeding women
- Participation in another clinical therapeutic study
- History of splenectomy or asplenia
- Hematologic malignancy
- Patients receiving chemotherapy
- Patients who will undergo or underwent an organ transplantation
- Patients with myasthenia gravis, glucose-6-phosphate dehydrogenase (G6PD) deficiency, or tuberculosis
- Patients who are or will be treated by plasmapheresis or hemodialysis
- Patient with a creatinine clearance of <30 or serum creatinine levels of >169 µmol/l
- Patients with a known hereditary complement deficiency
- Patients allergic to eculizumab, proteins derived from mouse products or other monoclonal antibodies
- Patients allergic to (prophylactic) antibiotic treatment for Neisseria meningitidis (quinolones or ceftriaxone)
- If on admission it is likely that the aneurysm can only be treated with extracranial-intracranial bypass surgery
- If based on head imaging, it will be unlikely that CSF can be obtained at day 3 after ictus
- Patients with an ongoing infection on admission which is not appropriately treated
- Patients who were treated >4 times with antibiotics during the last year
- Patients on immunosuppressive therapy

Legends: SAH= subarachnoid hemorrhage; CT= computed tomography; CTA= computed tomography angiography; DSA= digital subtraction angiography; UMC Utrecht= University Medical Center Utrecht; CSF= cerebrospinal fluid.

Table 2. Secondary outcomes

1. The occurrence of AEs and SAEs. Blinded assessment of infections will be performed by an expert panel consisting of a microbiologist and an infectious disease specialist

2. Blood and CSF parameters of inflammation

3. Eculizumab concentration in blood and CSF

4. Daily neurological condition measured by the GCS

5. Neurological condition measured by the NIHSS- and WFNS score on day 14 after ictus. If the patient is discharged earlier, the NIHSS- and WFNS score will be performed before discharge

6. Cerebral infarction defined as infarction identified on brain MRI after exclusion of procedure-related infarctions[17]

7. Cognition measured by the MoCA

8. Quality of life measured by the EQ-5D-5L questionnaire

9. Functional outcome measured by the mRS score. Telephone interviews will be conducted by a qualified person who is blinded for allocation.

Legends: AEs= adverse events; SAEs= serious adverse events; CSF= cerebrospinal fluid; GCS= Glasgow Coma Score; NIHSS= National Institutes of Health Stroke Scale; WFNS= World Federation of Neurosurgical Societies; MRI= Magnetic Resonance Imaging; MoCA= Montreal Cognitive Assessment; mRS= modified Ranking Scale.

Figures

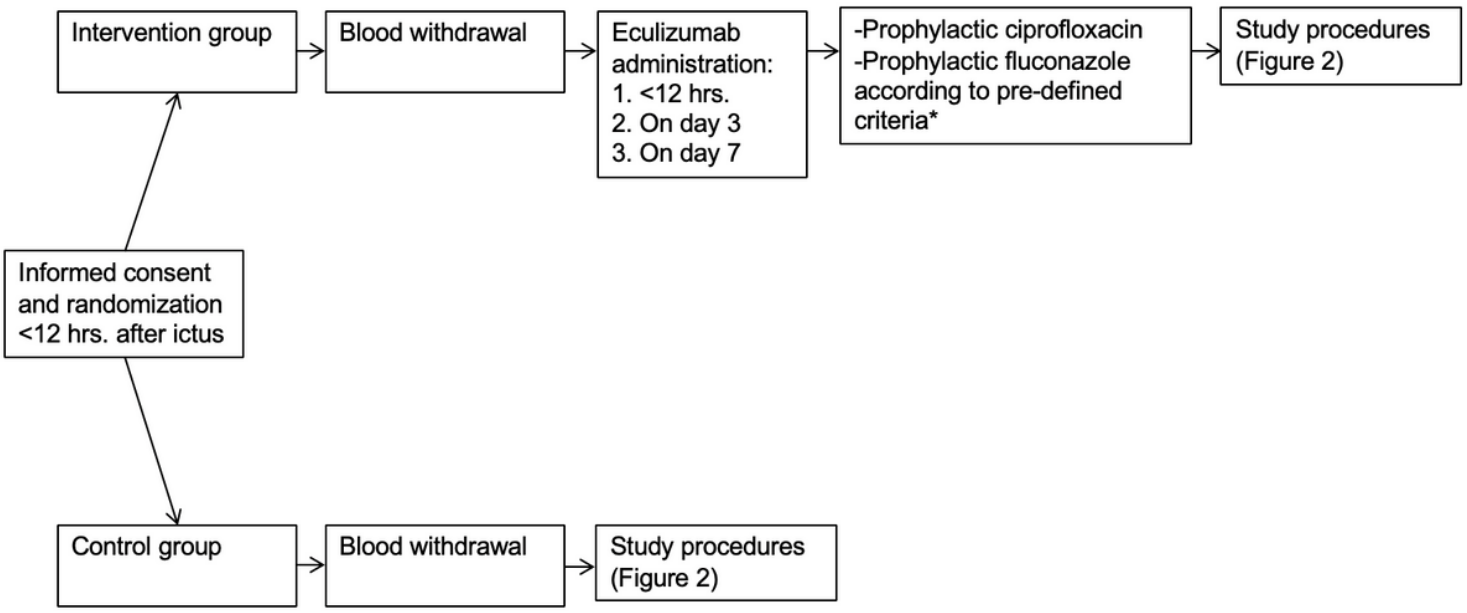

Figure 1

Treatment allocation. Hrs. = hours *Patients in the intervention group with a central line or an external ventricular shunt and a positive fungal or yeast culture will receive prophylactic fluconazole in addition to prophylactic ciprofloxacin.

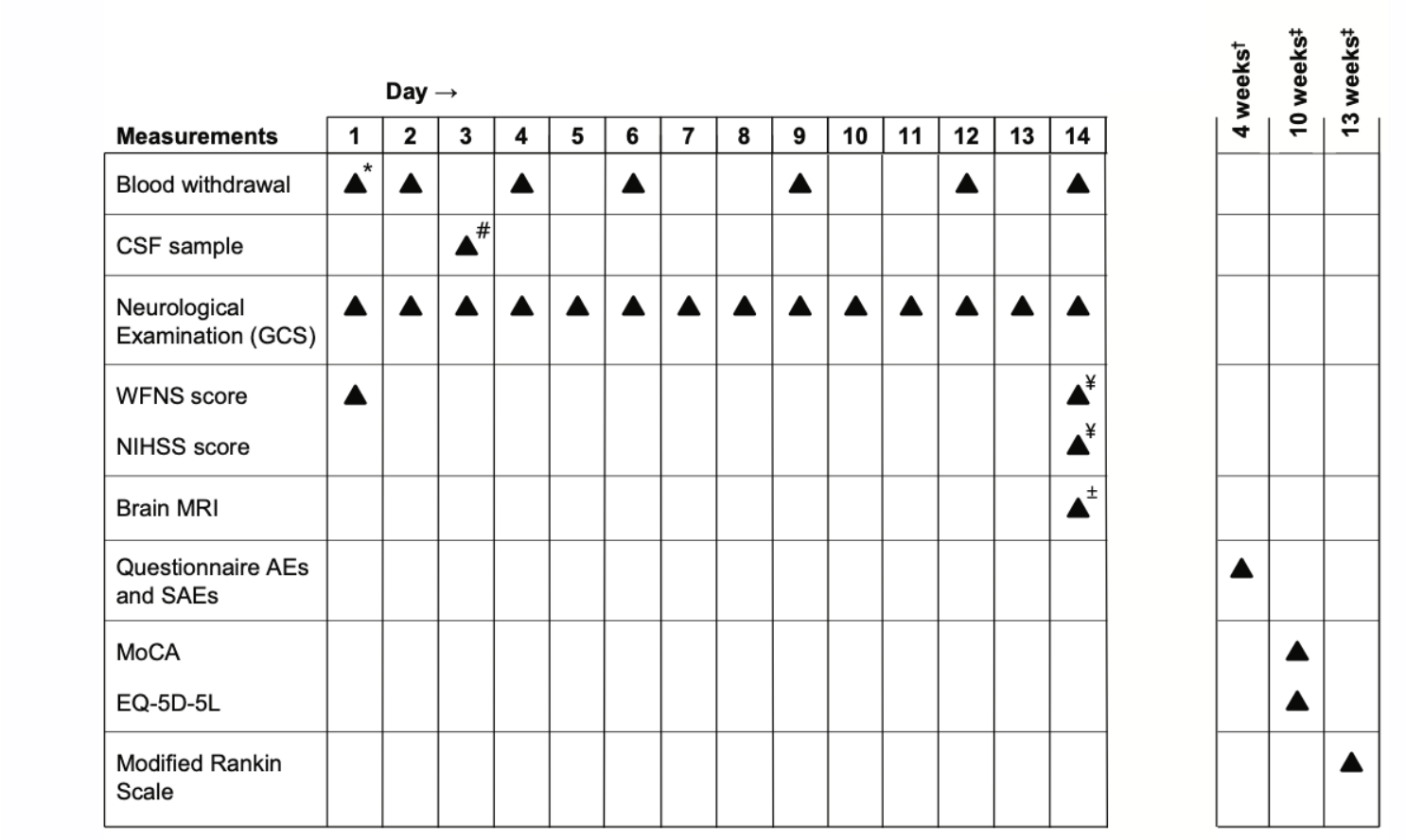

Figure 2

Study procedures. CSF= cerebrospinal fluid; GCS= Glasgow Coma Score; WFNS= World Federation of Neurosurgical Societies; NIHSS= National Institutes of Health Stroke Scale; MRI= Magnetic Resonance Imaging; AEs= adverse events; SAEs= serious adverse events; MoCA= Montreal Cognitive Assessment and; EQ-5D-5L= Standardized instrument for use as a measure of health outcome. * Blood withdrawal will be performed before the first eculizumab administration; # CSF will be obtained before the second eculizumab administration; ¥ If the patient is discharged earlier, the NIHSS- and WFNS score will be performed before discharge; If the patient is discharged earlier or clinically unstable, the MRI will be performed before discharge or postponed to a maximum of 28 days after ictus after which the MRI will be cancelled. If day 14 is during the weekend, the MRI will be performed before or after the weekend; † +/- one week ‡ +/- two weeks.

Supplementary Files

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