CLINICAL STUDY PROTOCOL

Open-label, phase II, single arm study to evaluate the safety, immunogenicity, pharmacokinetics and efficacy of recombinant human C1 inhibitor for the treatment of acute attacks in pediatric patients with hereditary angioedema, from 2 up to and including 13 years of age

Short title: Safety of Ruconest in 2-13 year old HAE patients

C1 1209

PROTOCOL TITLE: Open-label, phase II, single arm study to evaluate the safety, immunogenicity, pharmacokinetics and efficacy of recombinant human C1 inhibitor for the treatment of acute attacks in pediatric patients with hereditary angioedema, from 2 up to and including 13 years of age

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Short title	Safety of Ruconest in 2-13 year old HAE patients
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AUTHORIZED SIGNATURE PAGE AND AGREEMENT OF GCP COMPLIANCE

All parties involved in this study are committed to conducting the study in compliance with the protocol, ICH Good Clinical Practice Guidelines and the applicable regulatory requirements.

For approval on behalf of the Sponsor: Pharming Technologies BV
Darwinweg 24
2333 CR Leiden
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Name and function	Signature	Date
[redacted] Project Manager Pharming Technologies B.V.		11 Jun 2014
[redacted] Medical Director Pharming Technologies B.V.		11 Jun 2014
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INVESTIGATOR'S AGREEMENT

Open-label, phase II, single arm study to evaluate the safety, immunogenicity, pharmacokinetics and efficacy of recombinant human C1 inhibitor for the treatment of acute attacks in pediatric patients with hereditary angioedema, from 2 up to and including 13 years of age

The information contained in this document is CONFIDENTIAL and, except to the extent necessary to obtain informed consent, may not be disclosed unless such disclosure is required by government regulation or state/local customs or law. Persons to whom the information is disclosed must be informed that the information is CONFIDENTIAL and not be further disclosed by them.

By my signature below I agree to conduct this clinical trial in accordance with Good Clinical Practice, the Declaration of Helsinki, government regulations and state/local customs or laws, including those applying to institutional/ethics review and informed consent. I have read the Investigator's Brochure and protocol. I agree to ensure the confidentiality of my patients; however I agree to make available to the CROs, the Sponsor of this clinical trial, and relevant regulatory authorities, my patients' medical records. I am aware of my responsibilities as investigator.

Name	**Signature**	**Date**
Investigator:		

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2 LIST OF ABBREVIATIONS

AAE	Acquired angioedema
ACE	Angiotensin converting enzyme
ADR	Adverse Drug Reaction
AE	Adverse event
AEOSI	Adverse event of Special Interest
ALT	Alanine transaminase
Anti-HRI	Antibodies against host related impurities
Anti-rhC1INH	Antibodies against recombinant human C1 inhibitor
AST	Aspartate transaminase
BW	Body weight
C1INH	C1 inhibitor
CA	Competent authority
CRF	Case report form
CRO	Clinical research organization
CT	Computed tomography
CV	Curriculum Vitae
EC	Ethics committee
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
EudraCT	European drug regulatory affairs clinical trials
FFP	Fresh frozen plasma
GCP	Good clinical practice
HAE	Hereditary angioedema
HRI	Host related impurities
i.v.	Intravenous
IB	Investigator's Brochure
IC	Informed consent
ICF	Informed consent form
ICH	International Conference on Harmonization
IMP	Investigational medicinal product
IMPD	Investigational medicinal product dossier
IRB	Investigational review board
IS	Investigator score
ITT	Intention to treat
LDH	Lactate Dehydrogenase
MASP2	Mannan-associated serine protease 2
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration

MCV	Mean corpuscular volume
OPL	Oro-pharyngeal-laryngeal
OLE	Open label extension
OTC	Over the counter
pdC1INH	Plasma-derived C1 Inhibitor
PD	Pharmacodynamics
PK	Pharmacokinetics
PP	Per-protocol
QA	Quality assurance
RBC	Red blood cell count
RCT	Randomized controlled trial
rhC1INH	Recombinant human C1 inhibitor
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
Serpin	Serine protease inhibitor
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment emergent adverse event
TEQ	Treatment Effect Questionnaire
TTBR	Time To Beginning of Relief
TTMS	Time To Minimal Symptoms
VAS	Visual analog scale
WBC	White Blood Cell count
WFI	Water for injection

3 SYNOPSIS

Rationale:

The recombinant human C1 inhibitor (rhC1INH) clinical trials have evaluated 424 attack treatments in 155 HAE patients. The clinical and laboratory data demonstrate that rhC1INH represents an effective and well tolerated therapeutic option for the treatment of acute angioedema attacks in HAE patients.

C1INH deficiency is present at birth, and symptoms usually become apparent in the first or second decade of life. Patients typically experience minor swelling in childhood that may go unnoticed, with increased severity of symptoms around the time of puberty.

As a genetic disease, acute attacks of hereditary angioedema have the same pathophysiology in children and adults. Also as in adults, treatment with plasma-derived C1INH has been the standard of care for acute angioedema attacks in children for several decades. Recently, Ruconest was approved in Europe for the treatment of acute angioedema attacks in adults.

In previous studies, adolescents have been exposed to Ruconest without concerns about either efficacy or safety. However, no clinical trial data are available related to Ruconest exposure in patients under 14 years of age.

The main focus of this study is to collect data on safety and to identify eventual potential risks in pediatric HAE patients, to whom the product might be administered.

In this Phase II, open-label study, the aim is to assess the safety and efficacy of Ruconest for the treatment of acute HAE attacks in patients, from 2 up to and including 13 years of age.

Results of this clinical study will help evaluating the clinical benefit of Ruconest in this specific age group.

Objectives:

To assess the clinical safety, immunogenicity, and tolerability of Ruconest in the treatment of acute angioedema attacks in 2-13 year old HAE patients.

To assess the pharmacokinetics (PK) and pharmacodynamics (PD) of Ruconest in the treatment of acute angioedema attacks in 2-13 year old HAE patients.

To assess the efficacy of Ruconest in the treatment of acute angioedema attacks in 2-13 year old HAE patients.

Study design:

An open-label, non-comparative phase II, multinational, multi-center clinical study.

HAE patients from 2 up to and including 13 years of age will be screened for eligibility and enrolled.

Patients will be eligible for treatment with Ruconest if they present to the clinic within 5 hours of onset with an acute attack of at least moderate severity (Investigator Symptoms score (IS) of at least 3) without signs of spontaneous regression.

The patient will remain in hospital and closely monitored in the study center for at least 4 hours after study medication administration.

For safety evaluation, prior to and at Day 28 after study medication administration, a blood sample will be drawn for routine laboratory parameters. In addition to monitoring of vital signs (prior to and 30 min, 1, 2 and 4 hours after study medication administration), an ECG will be recorded prior to and between 30 minutes and 2 hours after study medication administration.

The evolution of the acute angioedema attack will be monitored by the administration of patient questionnaires (Visual Analog Scale (VAS) and Treatment Effect Questionnaire (TEQ)) at 30 min, 1, 2, 4, 8, and 24 hours, and the Investigator Score (IS) at 30 min, 1, 2 and 4 hours post study medication administration.

For PK/PD evaluation, blood samples will be collected prior to the first treated attack, and at 5 minutes and between 2 and 4 hours following the first study medication administration.

Four (4) hours after study medication administration the patient may be discharged from the clinic if the investigator judges the patient's condition well enough. The investigator will schedule a telephone contact at 24h (± 4 hours) after study medication administration. Follow up visits are planned at D28 (±3 days) and D90 (±7 days).

Multiple attacks can be treated, provided there is a minimum 24 hour interval between subsequent treated attacks and with a maximum of 10 attacks in the study, and as long as anti-rabbit epithelium (dander) IgE testing remains negative. Patients can therefore be treated beyond the age of 13.

Study population:

Patients from 2 up to and including 13 years of age, suffering from hereditary angioedema (baseline plasmatic levels of C1INH activity < 50%).

The criteria for the diagnosis of HAE will consist of a medical history confirmed by laboratory investigations.

Intervention:

One intravenous (i.v.) injection of Ruconest at the dose of 50 U/kg, for patients up to 84 kg; one i.v. injection of Ruconest at the dose of 4200U (2 vials) for patients of 84 kg body weight or greater.

The reconstituted solution should be administered as a slow i.v. injection over approximately 5 minutes. A second dose can be provided, at the investigator’s discretion, in case of insufficient therapeutic response. However, not more than two doses should be administered within 24 hours.

Main study parameters:

Safety and tolerability by standard criteria (vital signs, ECG, adverse events, routine laboratory safety parameters and immunogenicity (anti-host related impurities (HRI) and anti C1INH antibodies)

Efficacy parameters and endpoints (time to beginning of relief, time to minimal symptoms, time to complete resolution)

Pharmacokinetic and pharmacodynamic parameters (C1INH activity and C4 in plasma) during treatment for the first attack.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

All study-related procedures must be hosted in a familiar environment in facilities selected for childcare: the staff must be trained in communicating to and looking after (young) children and their legal representatives. All study-related procedures (e.g. blood samplings) will be optimized and modeled in order to minimize risk and distress. Age appropriate information will be given to the child and his/her representatives prior to any investigations or procedures. Eventual changes in the procedures will be announced to them well in advance.

The level of risk may evolve over time, during the study and with evolving knowledge; as a consequence, Pharming Technologies B.V. (the Sponsor) will continuously monitor this aspect. By regularly reviewing accumulated outcome data from the pediatric clinical trial program the continuing safety of current participants and those yet to be enrolled will be ensured, as well as the continuing validity and scientific merit of the study.

To date, the only significant clinical risk associated with Ruconest is the possibility of allergic reaction. To reduce the risk of a drug-induced allergic reaction, all study patients are tested for IgE against rabbit epithelium (dander). Only patients who have been shown to have negative results for such test should be treated with Ruconest.

4 INTRODUCTION AND RATIONALE

4.1 Introduction

HAE is a rare genetic disorder caused by a deficiency in functional endogenous C1INH activity with an estimated prevalence of 1 out of 50,000[1]. There is no known difference in its prevalence across ethnic groups[1, 2] or gender. In most patients, clinical symptoms begin in childhood or adolescence; the mean age at onset of the disease is 11.2 years[3]. The European Register of Hereditary Angioedema, following a survey of 1168 HAE patients from 10 European Union (EU) states, reported a median age of symptom onset of 11 years (range 0 to 86 years), and a median age of HAE diagnosis of 26 years (range 0 to 90 years)[4].

Over 150 different mutations of the affected C1INH gene causing HAE have been described, with all mutations resulting in reduced C1INH activity[1]. All these mutations result in either HAE type I or HAE type II. In HAE type I, there are reduced circulating levels of the C1INH protein and C1INH activity in patients' plasma. The median level of C1INH activity in individuals with HAE type I is about 20% of normal. In HAE type II, a dysfunctional mutant C1INH protein with no or reduced activity is transcribed from the mutated gene. This leads to reduced C1INH activity, with C1INH antigenicity levels that are normal or increased. The clinical manifestations of HAE type I and II are the same, therefore, in clinical practice, there is no distinction made between HAE types I and II. In untreated patients, a diagnosis of HAE is confirmed by the presence of reduced C1INH activity levels and low levels of plasma complement component 4 (C4).

The frequency of acute angioedema attacks varies widely across the HAE population. Patients have reported as few as 0 and as many as 50 attacks per year. In one large case series, 31% of patients reported <1 attack/year, 23% between 1-5 attacks/year, and 46 % ≥6 attacks/year. The frequency of attacks is not associated with the specific genetic mutation causing HAE, or with circulating C1INH activity or complement levels. Attacks may be precipitated by physical trauma, psychological stress, infection, estrogens or medications, notably ACE (angiotensin converting enzyme) inhibitors[5].

Patients with HAE experience episodic, recurrent acute attacks of angioedema, which are characterized by local swelling of soft tissues. Most attacks occur as single attacks, but 10 to 15% of attacks occur at multiple anatomical locations at the same time. Acute angioedema attacks may occur in the submucosal tissues of the gastrointestinal tract ("abdominal attacks"), vocal cords and larynx ("laryngeal attacks"), in the oropharynx ("oropharyngeal attacks"), and in the urogenital region ("urogenital attacks"). Attacks may also be localized in the subcutaneous tissues of the face ("facial attacks") or of the arms or legs ("peripheral attacks"). Observed differences between locations in time to relief appear to be related to differences in rate of fluid reabsorption, and not to any difference in pathophysiology. In the absence of treatment, the swelling due to an acute angioedema attacks worsens slowly but relentlessly over the first 24 hours, then gradually subsides over the subsequent 48 to 72 hours[1]. Acute angioedema attacks in HAE patients impair the quality of life, and can be fatal if the angioedema swelling occurs in the larynx and upper airways. An untreated attack can persist for up to five days, and may require hospitalization for treatment of dehydration and pain management or even intubation[6].

C1INH is a serine protease inhibitor (serpin), and is the major inhibitor of several complement proteases and contact-system proteases. Episodes of acute angioedema in patients with HAE result from an insufficient plasma level of functional C1 inhibitor, which allows the plasma proteolytic cascades such as complement and contact systems, to become more readily activated. This leads to episodic generation of several vasoactive substances such as bradykinin, which enhance vasopermeability. The clinical manifestation of this episodic increase in vascular

permeability is the occurrence of acute angioedema attacks[1]. Administration of a C1INH product normalizes C1INH activity and results in the control of complement and contact systems and the resolution of symptoms[7].

A summary of known and potential risks and benefits of the study product is included in section 9.4. More detailed information is available in the Investigators Brochure (IB). A summary of findings from non-clinical and clinical studies can be found below in sections 9.2 and 9.3.

4.2 Rationale

Ruconest has been approved in the 27 EU countries plus Norway, Iceland, Liechtenstein and Israel for the treatment of acute HAE attacks in adults. Only plasma-derived C1INH products are currently labeled for treatment of the pediatric population.

Results of this clinical study will help evaluate the balance of benefits and risks of Ruconest within this specific age group. A description and justification of the route of administration, dosage, dosage regimen, and treatment periods of the study medication formation is included in section 9.5.

Given the demonstrated efficacy in adults, the disease process being similar in adults and children and taking into account that Ruconest is a replacement therapy, the outcome of the therapy is likely to be comparable. An analysis of 16 adolescents ranging from 14-17 years of age having been treated for a total of 50 HAE attacks in previous studies does not suggest any difference in PK, clinical efficacy or safety, including immunosafety between this sub-population of adolescents and the overall population studied. Therefore, the current study is considered to be a clinical investigation with low risk while presenting the prospect of direct clinical benefit to the study participant.

In this Phase II open-label study, the primary aim is to assess the safety of Ruconest, especially when treating acute attacks of HAE, in pediatric patients from 2 up to and including 13 years of age.

5 OBJECTIVES

Primary Objective:

- to assess the clinical safety, immunogenicity and tolerability of Ruconest in the treatment of acute angioedema attacks in 2-13 year old HAE patients

Secondary Objectives:

- to assess the pharmacokinetics (PK) and pharmacodynamics (PD) of Ruconest in the treatment of acute angioedema attacks in 2-13 year old HAE patients
- to assess the efficacy of Ruconest in the treatment of acute angioedema attacks in 2-13 year old HAE patients

6 STUDY DESIGN

This study is an open-label, Phase II, non-comparative clinical study in pediatric patients from 2 up to and including 13 years of age, with a confirmed diagnosis of HAE.

The study will continue until at least 20 patients have been administered with Ruconest for at least one acute HAE attack.

Patients will be eligible for treatment with Ruconest if they present to the clinic within 5 hours of onset with an acute attack of at least moderate severity IS of at least 3) without signs of spontaneous regression.

The patient will remain in hospital and closely monitored in the study center for at least 4 hours after study medication administration.

For safety evaluation, prior to and at Day 28 after study medication administration, a blood sample will be drawn for routine laboratory parameters. In addition to monitoring of vital signs (prior to and 30 min, 1, 2 and 4 hours after study medication administration), an ECG will be recorded prior to and between 30 minutes and 2 hours after study medication administration.

The evolution of the acute angioedema attack will be monitored by the administration of patient questionnaires (Visual Analog Scale (VAS) and Treatment Effect Questionnaire (TEQ)) at 30 min, 1, 2, 4, 8, and 24 hours, and the Investigator Score (IS) at 30 min, 1, 2 and 4 hours post study medication administration.

For PK/PD evaluation, blood samples will be collected prior to the first treated attack, and at 5 minutes and between 2 and 4 hours following the first study medication administration.

Four hours after study medication administration the patient may be discharged from the clinic if the investigator judges the patient's condition well enough. The investigator will schedule a telephone contact at 24h (± 4 hours) after study medication administration. Follow up visits are planned at D28 (±3 days) and D90 (±7 days).

Multiple attacks can be treated, provided there is a minimum 24 hour interval between subsequent treated attacks and with a maximum of 10, and as long as anti-rabbit epithelium (dander) IgE testing remains negative. Patients can therefore be treated beyond the age of 13.

A schedule of trial assessments can be found in Appendix A.

If a new attack is treated before completing the follow-up visits for the previous attack, the D28 or D90 visit schedule will be reset based on the most recent treatment. Multiple attacks can be treated, provided there is a minimal interval of 24 hours between subsequent treated attacks.

7 STUDY POPULATION

7.1 Population (base)

Male and female patients, from 2 up to and including 13 years of age with a clinically suspected and/or confirmed diagnosis of HAE will be recruited for this study. Patients will be identified and invited to participate by the investigators at the respective study centers, if possible in close collaboration with the existing HAE Associations and/or other patient organizations and/or other referring physicians.

The criteria for the diagnosis of HAE will consist of a medical history supported by central laboratory investigations. The medical history of patients may include:

- self-limiting, non-inflammatory subcutaneous angioedema, without urticaria, often recurrent and often lasting more than 12h and/or
- recurrent self-remitting abdominal pain without clear organic etiology, often recurrent and often lasting more than 6h and/or
- recurrent laryngeal edema
- family members with recurrent angioedema and/or abdominal pain and/or laryngeal edema

A central laboratory will confirm the diagnosis of HAE, defined as:

- <50% of normal levels of C1INH activity

Secondary laboratory data supporting the diagnosis of HAE are:

- not abnormally low levels of C1q
- absence of C1INH auto antibodies (anti-C1INH IgM and IgG)

The results of secondary laboratory parameters in plasma collected at screening serves to exclude from the study patients with evidence for acquired angioedema (AAE) (by a level of C1INH activity <50% of normal, together with a low level of C1q and/or presence of anti-C1INH antibodies).

Patients will be qualified for recruitment in advance and will be instructed to report to the clinical centre as quickly as is safely possible at the onset of symptoms of an acute attack. The diagnosis of an HAE attack will be made by the investigator based on patient's and physician's experience and judgment, and the results of physical examination.

The results of non-invasive diagnostic procedures deemed necessary to exclude other pathologies (e.g. cholecystitis, pancreatitis, nephrolithiasis, appendicitis, peritonitis, salpingitis, trauma, allergies) and/or the results of study specific safety laboratory investigations should be recorded in the CRF. The results of the tests will thus serve to further ensure that a correct diagnosis of an HAE attack has been made.

Patients who present to a study center within 5 hours of onset of an attack of angioedema will be evaluated for eligibility by the investigators, based on the clinical presentation of the patient's angioedema symptoms. The investigator will use a scoring tool for each affected anatomical location (see form in appendix D), based on a variety of symptoms associated with swelling (such as pain and/or respiratory complaints and/or abdominal discomfort and/or urination problems depending on the location). An HAE attack in a patient with HAE will be considered eligible if the investigator's score at this location at this time of evaluation is at least 3 (moderate symptoms)

and there is no evidence of regression of angioedema symptoms prior to treatment. A severity score of 4 corresponds to severe symptoms, whereas the highest score of 5 represents a life-threatening situation.

If the patient has arrived at the study center within the 5 hour time-frame, but the IS score is less than 3 at first evaluation by the investigator, then additional IS scores may be collected within the 5 hour time-frame from the onset of symptoms. If a significant increase in symptom severity at any localization occurs, the time-point within the 5 hour time-frame at which the IS score is at least 3 will be further referred to as the Presentation time point.

7.2 Inclusion criteria

Screening

- From 2 up to and including 13 years of age
- Clinical and laboratory confirmed diagnosis of HAE (baseline C1INH activity <50% of normal)
- Signed written informed consent (parental permission) signed by the legal guardian(s)

Treatment

- Clinical symptoms of an acute HAE attack
- Onset of eligible symptoms within 5 hours from the moment at which medical evaluation to determine eligibility has occurred
- IS score for at least one anatomical location at the time of initial evaluation of at least 3 (moderate severity or greater) without signs of spontaneous regression
- 24h or more have passed since the patient's last study treatment

7.3 Exclusion criteria

Screening

- A diagnosis of acquired C1INH deficiency (AAE)
- A medical history of allergy to rabbits or rabbit-derived products (including rhC1INH, antisera), or positive anti-rabbit epithelium (dander) IgE test (cut off>0.35 kU/L in ImmunoCap® assay (Phadia, Sweden) or equivalent)
- Treatment with investigational drug in another clinical study in the last 30 days
- Any clinical significant abnormality in the physical examination and/or the routine laboratory assessments, that in the opinion of the Investigator makes the patient unsuitable for participation in the study
- Patient or legal guardian whose decision to participate might be unduly influenced by perceived expectation of gain or harm by participation, such as patient or legal guardian in detention due to official or legal order
- Any condition or treatment that in the opinion of the investigator might interfere with the evaluation of the study objectives

Treatment

- Any changes since screening that would exclude patient based on above exclusion criteria.
- 10 HAE attacks were previously treated with study medication.
- Suspicion for an alternate explanation of the symptoms other than an acute HAE attack.
- Use of any disallowed concomitant medication since onset of acute HAE attack (see Section 8.2.1).
- Positive pregnancy test (urine or serum)

7.4 Differential Diagnosis of Abdominal Pain

The investigator will diligently consider the patient's history, review of systems, physical examination, and results of all available laboratory and non-invasive diagnostic tests to establish the likelihood that HAE is or is not the cause of the symptoms of the attack with which the patient acutely presents. It is recognized that the investigator may, in good faith, consider that HAE is responsible for a given attack, and if other criteria for eligibility are met, treat the patient within the intended 5 hr window. However, subsequently obtained laboratory test results may suggest that symptoms were not due to HAE. If these results are not available, the study medication will be administered as scheduled. It is recognized that results of these tests, and/or additional diagnostic testing may suggest that the symptoms are not due to HAE. Criteria suggesting alternate diagnoses may include:

1. Total WBC above 20,000 or (segs + bands) above 15,000
2. Fever (oral temp > 38° C)
3. Amylase > 2.5 x upper limit of normal for that laboratory
4. Free air under the diaphragm on KUB X-ray.
5. Blood in urine or stool in patient in whom these tests were negative at the time of screening
6. Definitive diagnosis established by any other means, including surgical intervention

In case the diagnosis of an acute HAE attack is doubted, particularly in the presence of fever, it will be important to consider diagnoses other than HAE (e.g. acute abdominal emergencies), and perform additional examinations and tests to evaluate other possibilities. Ultrasound and/or other imaging procedures may be performed whenever necessary, at the discretion of the investigator, but should not be allowed to interfere with initiating treatment within 5 hr of onset. If another diagnosis is reached (after a patient has been treated); data for that patient will not be excluded from the intention to treat (ITT) analysis or safety and immunogenicity evaluations.

7.5 Sample size calculation

No formal sample size was calculated; it was anticipated that an overall sample of at least 20 completed patients is a sufficient dataset to meet the objectives of the study; this target number of patient is based on feasibility and agreed with the Paediatric Committee of the European Medicines Agency. The enrolment of patients will continue until at least 20 different patients are treated for at least one acute HAE attack, and the Sponsor determines that sufficient data have been collected on repeat treatments.

8 TREATMENT OF PATIENTS

8.1 Intervention

Patients up to 84 kg will receive one i.v. injection of Ruconest at a dose of 50 U/kg. The reconstituted solution should be administered as a slow i.v. injection over approximately 5 minutes. Patients of 84 kg body weight or greater will receive one i.v. injection of Ruconest at the dose of 4200 U (2 vials).

At the discretion of the investigator and depending upon the patient's clinical response, an additional dose may be given to patients following their initial dose as specified above. Not more than two doses should be administered within 24 hours.

8.2 Co-medication

After recruitment, the patients will in principle continue on the kind and dose of prophylactic medication prescribed for HAE that they are currently using. After recruitment, changes in kind and dose of maintenance therapy for HAE are only allowed after consultation with the investigator.

The investigator, if possible in collaboration with the Sponsor, will evaluate the impact of any other drug treatment deemed necessary for HAE and/or an intercurrent condition after recruitment and/or after treatment with study medication.

All concomitant medications (prescription and/or over-the-counter medications, herbal medications, preventative vaccines, vitamins and food supplements) and procedures must be recorded in the Case Report Form (CRF). A description of the type of drug or procedure, the amount, duration, reason for administration of drug, and the outcome of any procedures must be documented. Adverse Events (AEs) related to the administration of a concomitant medication or the performance of a procedure must also be documented on the appropriate AE page of the CRF.

8.2.1 Disallowed Concomitant Medication

Patients presenting with attacks who already have received any medication since the onset of the attack likely to interfere with the evaluation of efficacy and safety will **not be eligible** for treatment for this attack. These treatments include:

- Narcotics
- plasma-derived C1 inhibitor
- Fresh frozen plasma
- Bradykinin receptor antagonist
- Analgesics
- Anti-emetics
- Non Steroidal Anti-Inflammatory Drugs (NSAIDs)

As an exception, for the patient's comfort, paracetamol may be taken, but not within 45 minutes before the administration of study medication. Any change in current and optional maintenance therapy (androgens and/or anti-fibrinolytics) from the time of the onset of the current attack is not allowed. The dosage of androgens and anti-fibrinolytics may not be increased earlier than 24 hours after the treatment with study medication.

To minimize impact on the assessment of safety, treatment with other investigational products is not allowed from screening until 30 days after the patient's last follow-up visit has occurred.

8.2.2 Rescue medication

An aim of this open label study is to evaluate the safety and efficacy of Ruconest in symptomatic HAE. Therefore, it is recommended to consider administering another dose of Ruconest as a first attempt of rescue medication, which is consistent with the SPC instructions. The use of additional treatment which might interfere with the evaluation of efficacy and safety is not allowed (exclusion criterion) prior to treatment with study medication (see section 8.2.1) and after the administration of study medication for as long as possible up to 8 hours. As specified in section 10.1.3, taking such additional treatment (other than Ruconest) between the onset of the attack and prior to time of initial relief of symptoms, will qualify that attack as "treatment failure". However, appropriate and urgent medical need will not be ignored nor withheld: the use of narcotics or other standard procedures for the treatment of HAE after treatment with study medication will be allowed at the discretion of the investigator.

Situations in which the investigator may decide to install additional treatment and/or supportive measures after the administration of study medication include:

1. Clear progression of symptom severity, with or without the occurrence of symptoms at new angioedema locations.
2. Unacceptable degree of abdominal pain.
3. Symptoms indicating life is threatened (respiratory and/or cardiovascular compromise), i.e. symptoms requiring immediate additional treatment. Patients developing a life-threatening attack (e.g. a laryngeal attack with respiratory and/or cardiovascular compromise) after the administration of study medication must also be treated with any treatment procedure (including intubation, tracheostomy) deemed necessary.
4. AEs requiring specific treatment (e.g. in case of anaphylaxis).

Pain medication, anti-emetics and fluid replacement may be used in case of acute attack(s), if needed. In case of therapeutic failure, rescue therapies (such as pdC1INH) or fresh frozen plasma may be administered, according to local clinical standards.

All (escape) medications taken during acute episodes should be recorded on the CRF with indication, dose, timing and apparent response.

All above guidelines are meant to prevent extreme discomfort to the patient during the study and to allow adequate and appropriate action to be taken when necessary. Actions unequivocally pertinent to patient care should always take priority over study interests.

9 INVESTIGATIONAL MEDICINAL PRODUCT

9.1 Name and description of investigational medicinal product

Ruconest 2100 U powder for solution for injection contains the active substance recombinant human C1 inhibitor (rhC1INH; conestat alfa) which is purified from the milk of rabbits expressing the gene encoding for human C1 inhibitor (C1INH). Each vial contains 2100 units of rhC1INH. The amino acid sequence of the recombinant form is identical to that of human C1INH. C1INH is a single-chain plasma glycoprotein of molecular mass 73,650 that belongs to the super family of serine protease inhibitors in plasma.

C1INH is the only known inhibitor of activated subcomponents C1s and C1r of complement component 1 of the classical pathway of the complement system. In addition, C1INH inhibits the Mannan-Associated Serine Protease 2 (MASP2) of the lectin pathway of complement activation. Furthermore, it is the major inhibitor of activated factor XII, factor XI and kallikrein of the contact system in plasma.

For further information please refer to the Investigator's Brochure (IB) [13].

9.2 Summary of findings from non-clinical studies

9.2.1 Pharmacological characteristics

The inhibitory potency of rhC1INH towards the target proteases C1s, kallikrein, factor XIa and factor XIIa was found to be comparable with the inhibitory potency of human plasma-derived C1INH. In addition, no difference in inhibition of plasmin and thrombin was observed between rhC1INH and human plasma-derived C1INH.

9.2.2 Summary of animal experiments

Acute and repeated dose toxicology and pharmacology as well as local tolerance studies in rats, dogs and rabbits indicate that the preparation may be considered safe. rhC1INH was cleared more rapidly from the circulation of the rat and the dog in comparison with plasma-derived human C1INH. This faster clearance likely results from the differential and lower degree of glycosylation (e.g. less sialylation) in rhC1INH. For further information see the IB [13].

9.3 Summary of findings from clinical studies

The development program of rhC1INH comprises a total of 714 exposures to rhC1INH in 190 subjects. As can be seen from the table below, it not only concerns symptomatic patients for the treatment of acute attacks, but also healthy volunteers and asymptomatic patients. Each of these studies is separately discussed in this paragraph.

Study	Study Population	Number of Subjects[a]	Number of Administrations
1202/1203	Symptomatic HAE patients	14	21
1205 RCT	Symptomatic HAE patients	25	25
1205 OLE	Symptomatic HAE patients	62	168
1304 RCT	Symptomatic HAE patients	16	16
1304 OLE	Symptomatic HAE patients	57	194
Subtotal	***Symptomatic HAE patients***	***155***	***424***
1101	Asymptomatic HAE patients	12	24
1106	Healthy volunteer subjects	14	59
1207	Asymptomatic HAE patients	25	207
Total all studies	**All populations**	**190**	**714**

[a] Because some subjects participated in more than one study, the total number of subjects is smaller than the number reached by adding the number of subjects in each of the individual studies.

Study C1 1101-01

In Study C1 1101-01, administration of escalating doses of rhC1INH (6.25 to 100 U/kg) to 12 asymptomatic HAE patients resulted in dose-dependent increases of C1INH activity. Doses of rhC1INH of 50 U/kg and 100 U/kg were found to restore C1INH activity to normal levels (0.7 -1.3 U/mL), while doses of 25 U/kg and lower did not. After administration of 50 U/kg rhC1INH, a C_{max} of 1.36 U/mL is observed. Levels remain above 0.7 U/mL for approximately 2 hours and then subside to baseline levels in approximately 4 hours. rhC1INH was shown to be pharmacodynamically active in HAE patients through a dose-dependent decrease in the formation of C4b/c, the activation cleavage product of plasma complement C4. Doses of 100 U/kg and 50 U/kg increased mean normalized levels of C4 relative to baseline, and cleavage of C4 resumed once C1INH activity levels fell below 0.7 U/mL. Doses of rhC1INH of 25 U/kg or lower only resulted in a temporary, minimal elevation of C4 levels relative to baseline.

Studies C1 1202-01 and C1 1203-01

Exploratory efficacy Studies C1 1202-01 and C1 1203-01 were undertaken in 14 symptomatic HAE patients, treated for 21 acute angioedema attacks.

The treatment appeared safe and well-tolerated. No clinically significant AEs, changes in vital signs, safety laboratory parameters or antibody responses to C1INH or rabbit milk protein were observed. The PK findings from Study C1 1101-01 were confirmed in HAE patients treated with rhC1INH for an acute angioedema attack. Both patients and physicians evaluated treatment with rhC1INH as favorable compared to previous untreated attacks. The median time to the beginning of relief was 30 and 60 minutes, as reported by physicians and patients, respectively. The median time to minimal symptoms was 4 hours. All treated attacks resolved completely and no relapses occurred. No difference in response was observed on first and repeated treatments.

Study C1 1106-02

In Study C1 1106-02, fourteen healthy volunteers were enrolled to receive rhC1INH at 100 U/kg on five occasions with intervals of approximately 3 weeks between doses. Serial PK sampling was performed following the first, third and fifth administrations of rhC1INH. After administration of rhC1INH the levels of C1INH activity peaked at median times of about 20-30 minutes and thereafter declined to endogenous levels in about 8 to 12 hours. No difference in PK profiles was observed following the 1st, 3rd, and 5th administrations of rhC1INH.

One anaphylactic reaction occurred in an adult female participating in this study on first exposure to rhC1INH. This patient had a clinical history of rabbit allergy (not disclosed during the study pre-consent screening procedure). The patient was also subsequently found to have a clinically significant IgE antibody level against rabbit epithelium (dander) allergens.

No clinically significant changes in hematology, biochemistry, urinalysis, coagulation, vital signs or ECG parameters or physical examinations were noted in this study.

Study C1 1207

In Study C1 1207, an open-label exploratory phase II study, the safety and prophylactic effect of weekly administrations of rhC1INH was studied in asymptomatic HAE patients. Twenty-five patients were enrolled and treated with rhC1INH at 8 weekly administrations of 50 U/kg and followed up for 42 days after the last administration.

For the primary endpoint, the incidence of documented HAE attacks during the treatment period (breakthrough attacks), a median of 2 attacks over the 8 week period was found corresponding to an average of 0.25 attacks per week (range 0 to 1.5). This was lower than the reported history of HAE attacks over a two year period prior to entry in the study (0.6 attacks per week (range 0.5 – 4.5)).

Immunology testing revealed the presence of confirmed anti-rhC1INH antibodies in 2/25 patients, but no neutralizing antibodies. Confirmed antibodies against Host Related Impurities were found in 11/25 patients at any time point during the study without associated clinical symptoms. Thirty treatment-emergent adverse events were observed during the study in 13 patients, of which two were serious adverse events. Four adverse events, all mild in intensity, were considered possibly drug-related by the investigator. All other adverse events, including the serious adverse events, were assessed as not related to rhC1INH by the investigator. No events led to discontinuation of study medication.

No trends in laboratory values were observed. All out or range and clinical significant laboratory parameters were related to adverse events, HAE attacks or concomitant disease. In addition, no trends were observed for vital signs, physical examinations or ECG.

Study C1 1304-01 RCT

In randomized, double blind, saline controlled clinical Study C1 1304-01 RCT, the efficacy and safety of rhC1INH 100 U/kg in the treatment of acute angioedema attacks in HAE patients compared to saline was evaluated. Thirty-two patients were randomized and treated in the study. For the primary endpoint, time to beginning of relief, a statistically significant difference was found between 100 U/kg and saline solution groups (62 and 508 minutes, respectively; p=0.003). Treatment with rhC1INH was also statistically significant in reducing the time to minimal symptoms compared to using saline solution (480 and 1440 minutes, respectively; p=0.005). The

analysis of therapeutic failures supported the efficacy of rhC1INH by demonstrating that significantly fewer patients in the rhC1INH group had failure compared with patients in the saline solution dose group.

Treatment with rhC1INH did not result in changes in vital signs, ECG, routine clinical laboratory safety parameters, and no relevant antibody responses to C1INH or rabbit milk protein impurities were observed. No drug-related TEAEs were observed after treatment with rhC1INH.

Study C1 1205-01 RCT

In randomized, double blind, saline controlled clinical Study C1 1205-01 RCT, the efficacy and safety of rhC1INH 100 U/kg and 50 U/kg in the treatment of acute angioedema attacks in HAE patients compared to saline was evaluated. Thirty-eight patients were randomized and treated in the study. For the primary endpoint, time to beginning of relief, a statistically significant difference was found between 100 U/kg and 50 U/kg and saline solution groups (68, 122, and 258 minutes; $p=0.001$ and $p < 0.001$, respectively). The analysis of therapeutic failures supported the efficacy of rhC1INH by demonstrating that significantly fewer patients in the rhC1INH groups had failure compared with patients in the saline solution dose group.

The majority of patients experienced no TEAEs or TEAEs that were mild or moderate in severity in all treatment groups. No possibly, probably or definitely related to study treatment SAEs occurred during the study. Treatment with rhC1INH did not result in changes in vital signs, routine clinical laboratory, safety parameters, and no clinically relevant antibody responses to C1INH or rabbit milk protein impurities were observed.

Study C1 1304-01 OLE

The C1 1304-01 OLE study allowed the open label extension treatment of acute angioedema attacks in HAE. In this study, HAE patients could be treated multiple times for subsequent new acute angioedema attacks. The initial rhC1INH treatment was a fixed dose of a single 2100 U vial. This initial fixed dose could be followed by an additional one or two 2100 U vial(s) at the discretion of the investigator and depending on the patient's clinical response within 4 hours after administration of the initial treatment.

Fifty-seven HAE patients received a total of 194 OLE treatments of acute angioedema attacks.

Results of the primary endpoint, time to beginning of relief, showed a sustained efficacy of rhC1INH in repeated treatments for attacks 1 to 5 of 60, 65, 120, 60 and 61 minutes (Median time to beginning of relief using the overall VAS score). The apparent difference of the median time of attack 3 (120 min) is likely an artifact of the discrete assessment times, i.e. 60 minutes then 120 minutes, based on the fact that the 95% confidence intervals for time to beginning of relief for attack 3 were similar to the other four attacks analyzed.

Immunology testing revealed the presence of confirmed anti-rhC1INH antibodies in 4/57 patients at any time point during the study, but no neutralizing antibodies. Confirmed antibodies against Host Related Impurities were found in 3/57 patients at any time point during the study without associated clinical symptoms.

Twenty-seven patients (47%) reported at least one treatment emergent adverse event, most of them mild to moderate in severity. Three adverse events were considered severe, but not related to study medication. Four patients experienced possibly related TEAE, as determined by the investigator, but none of them required any treatment. There was no increase in the number of

TEAEs with repeated treatments. Two SAEs were reported and were both considered as not related. None of the TEAEs led to death or study medication discontinuation. Treatment with rhC1INH did not result in changes in vital signs, ECG, and routine clinical laboratory safety parameters.

Repeated treatments with rhC1INH appeared generally safe and well tolerated, and did not result in a change in adverse event profile or incidence.

Study C1 1205-01 OLE

The C1 1205-01 OLE study allowed the open label extension treatment of acute angioedema attacks in HAE. In this study, HAE patients could be treated multiple times for subsequent new acute angioedema attacks. The initial rhC1INH treatment was 50 U/kg with an option for an additional 50 U/kg at the discretion of the investigator and depending on the patient's clinical response within 4 hours after administration of the initial treatment.

Sixty-two HAE patients received a total of 168 OLE treatments of acute angioedema attacks.

Results of the primary endpoint, time to beginning of relief, showed a sustained efficacy of rhC1INH in repeated treatments for attacks 1 to 5 of 63, 61, 37, 51 and 67 minutes (Median time to beginning of relief using the overall VAS score). The 95% confidence intervals of the repeated attacks are overlapping (30-124 min).

Immunology testing revealed the presence of confirmed anti-rhC1INH antibodies in 2/62 patients at any time point during the study, but no neutralizing antibodies. Confirmed antibodies against Host Related Impurities were found in 2/62 patients at any time point during the study without associated clinical symptoms.

Thirty-nine patients (63%) reported at least one treatment emergent adverse event, most of them mild to moderate in severity; 8 TEAEs were possibly related to treatment according to the investigator's opinion. Seven adverse events were considered severe in intensity, none of them assessed by the investigator as treatment related. Twenty-two SAEs were reported in 10 patients. Thirteen SAEs were HAE attacks in 7 patients. All SAEs resolved and none was related to treatment in the investigator's opinion except for a "hypersensitivity reaction" which was considered possibly related to treatment. Laboratory data suggest that the event was not an IgE-mediated reaction.

Repeated treatments with rhC1INH showed continued efficacy and appeared generally safe and well tolerated.

Adolescent patients treated in the open label extension studies

In the open label extension studies mentioned above, enrolment of adolescent patients was allowed, and this subset of data was separately evaluated. A total of 16 patients have been exposed to rhC1INH for a total of 50 treatments of an acute HAE attack, ranging from 14 to 17 years of age at the time of treatment; 8 patients have been exposed to rhC1INH for at least three HAE attacks, 2 patients even up to 7 HAE attacks.

Due to the different dosing strategies, there was a range in treatment doses, varying from 25 U/kg to 100 U/kg. As for the adult population, PK-data in adolescents indicated that a dose of 50 U/kg will restore deficient levels of C1INH activity into the normal range (70% to 130%).

In the adolescent subpopulation, across studies the medians were 45 minutes for the TTBR. These results, irrespective of attack number, are consistent with the overall observed outcomes, and do not indicate a need for different dosing strategy in adolescents compared to the adult population.

Six patients (38%) reported at least one TEAE. All Adverse events were mild to moderate in severity. None of the recorded adverse events led to premature study discontinuation or death. Also, no Serious Adverse Events (SAE) were reported for these patients. No adverse effects were observed in vital signs, and none was observed suggesting hypersensitivity reactions or thrombogenicity. There were no treatment emergent antibodies.

This information does not suggest any difference in PK, clinical efficacy or safety, including immunosafety between this sub-population of adolescents and the overall population studied.

For detailed information of safety and effects of rhC1INH in each of the studies mentioned above, see the IB [13].

9.4 Summary of known and potential risks and benefits

9.4.1 Expected adverse events during the study

During clinical studies in symptomatic HAE patients, AEs during the study period may relate to the multitude of possible symptoms associated with an acute angioedema attack at one or more anatomical locations. In some cases it may be difficult to discern symptoms related to an attack with certainty from those related to the administration of rhC1INH.

The safety data analyses from 424 exposures to rhC1INH showed that rhC1INH at doses of 50 U/kg and 100 U/kg is generally safe and well tolerated (see IB for details). The adverse event profile found in the RCT analyses was similar for patients treated in the rhC1INH and saline treatment groups. The AE profile was the same across all rhC1INH doses analyzed. There was no increase in the incidence of TEAEs with higher rhC1INH dose, administration of additional rhC1INH doses for an attack, or with repeat treatment of subsequent attacks.

To date, the only significant clinical risk associated with rhC1INH is the possibility of allergic reaction to host-related impurities (HRI). Only patients who have been shown to have negative results for IgE antibodies against rabbit epithelium (dander) should be enrolled in the study.

It is notable that participation in clinical studies often results in headache, and also that hematoma and minor pain may occur as a result of the insertion of intravenous cannulas and venipunctures. Risks of these complications are minimized by use of experienced personnel.

9.4.2 Rare adverse events during the study

As with any medication, rare side effects cannot be excluded.

9.4.3 Antidotes

Specific antidotes against rhC1INH do not exist. For allergic reactions appropriate standard treatment regimens indicated by the clinical situation (e.g. epinephrine, antihistamines, corticosteroids, etc) will be administered.

9.5 Description and justification of route of administration and dosage

Ruconest has been developed and was approved in the 27 EU countries plus Norway, Iceland , Liechtenstein and Israel for the treatment of acute attacks of angioedema in adult patients with HAE due to C1 inhibitor activity deficiency. Because C1INH is a plasma glycoprotein, an intravenous route of administration has been chosen.

The efficacy of Ruconest was demonstrated in two independently conducted RCTs in North America (Study C1 1205-01 RCT) and in Europe (Study C1 1304-01 RCT). The current study will provide further data on the safety and immunogenicity of Ruconest at a dose of 50 U/kg when used for the treatment of acute angioedema attacks in 2-13 year old HAE patients.

Ruconest, at the dose of 50 U/kg body weight (and with a maximum of 4200 U), will be administered by slow intravenous injection over a period of approximately 5 minutes. This dose and method of administration appeared safe and well tolerated in previously treated patients with HAE, including 16 adolescent patients.

9.6 Preparation and labeling of Investigational Medicinal Product

The Ruconest treatment solutions will be prepared from vials containing 2100 U of lyophilized rhC1INH. Each vial will be labeled and packed in a box. Ruconest will be labeled in accordance with regulatory requirements as follows:

Sample of outer package label:

Recombinant human C1 Inhibitor (rhC1INH) **Batch Nr**.: XXXXX **- Expiry date:** XX/XXX/XXXX Lyophilized powder. For intravenous infusion. 2100 U/vial - Reconstitute with 14 mL WFI Property of Pharming Technologies B.V. Darwinweg 24, NL-2333 CR, Leiden The Netherlands - T: 0031 (0) 715247400
For clinical trial use only Protocol Nr.: C1 1209 To Be Used By Qualified Investigator Only Investigator: XXXXXXX Store at 2-25 °C

Sample of vial label:

	Recombinant human C1 Inhibitor (rhC1INH) Protocol Nr.: C1 1209 Property of Pharming Technologies B.V. - T: 0031 (0) 715247400 Darwinweg 24, NL-2333 CR, Leiden, The Netherlands **Batch Nr.:** XXXXX **- Expiry date** XX/XXX/XXXX Lyophilized powder. For intravenous infusion. 2100 U/vial - Reconstitute with 14 mL WFI **For clinical trial use only** To Be Used By Qualified Investigator Only Investigator; XXXXXXX Store at 2-25 °C (35.6-77°F)	**Clinical trial C1 1209** Pharming Technologies B.V. Subject initials: X X X Subject number: X X X X X

Each vial containing the lyophilized material will be reconstituted with 14 mL water for injection (WFI) prior to use.

The preparations will be dispensed at the investigator's clinical center or by the hospital pharmacy of each investigational center with labels containing information similar to the sample label above.

For detailed pharmaceutical instructions, see Appendix E.

9.7 Drug accountability

All study medication will be stored at the responsible investigator's clinical center and/or the Department of Clinical Pharmacy of the various hospitals. Drug accountability will be performed and monitored according to ICH-E6(R1) "Guideline for Good Clinical Practice".

As this study involves i.v. administration of the study medication administered by study personnel, patient compliance measures are not necessary. Study medication will be administered under direct supervision, according to the center's standard operating procedures. Two members of the center clinical staff (who should include at least one physician) will check the medication label for agreement with the study number and the patient's identity. Information with respect to volume of prepared and infused study medication will be documented.

10 METHODS

10.1 Study parameters

10.1.1 Main study parameter

The primary objective is the assessment of safety and tolerability.

This will be evaluated by recording of adverse events (spontaneously reported by the patient or observed by the investigator.

Immunogenicity (for all attack treatments) will be evaluated by the following parameters:

- antibodies against host related impurities (anti-HRI)
- antibodies against recombinant human C1INH (IgG and IgM) anti-rhC1INH)
- IgE antibodies against rabbit epithelium (anti rabbit epithelium IgE)

10.1.2 Secondary study parameters

At the first attack, pharmacokinetic and pharmacodynamic parameters will be evaluated for:

- C1INH activity and C4.

The following parameters will be determined: baseline level; C_{max}; dose-normalized C_{max}; T_{max}; AUC above baseline; dose-normalized AUC; clearance; volume of distribution; elimination half-life ($t_{½}$); V_{max}; K_m.

Efficacy will be evaluated by

- time to beginning of relief assessed by using the overall severity VAS, defined as the first time point with a decrease of at least 20 mm with respect to baseline at any eligible location, with persistence at the next time point (see section 10.2.3)
- time to minimal symptoms assessed by using the overall severity VAS, defined as the first time point at which the overall severity VAS falls below 20 mm for each assessed location
- time to beginning of relief assessed by using the IS-score defined as the first time point with a decrease of at least 1 point on the IS-score (investigator assessment) compared to baseline, at any eligible location.
- time to minimal symptoms assessed by using the IS-score, defined as the first time point at which the IS-score is less than or equal to 1 point for all assessed locations

All terms are defined in the List of Definitions in Appendix F. See section 10.2.3 for a detailed description of these efficacy parameters.

10.1.3 Other study parameters

Safety variables after single and repeat treatment that will be subject to exploratory analysis:

- vital signs
- ECG
- Routine clinical laboratory parameters

Efficacy variables that will be subject to exploratory analysis:

- time to beginning of relief based on questions 1 and 2 of the TEQ, defined as the first time-point at which Question 1 is answered as a little better, better or much better and Question 2

is answered as yes for the most severe eligible location, with persistence of these responses at the next time point.

- time to minimal symptoms on the basis of Question 3 of the TEQ, defined as the first time-point at which Question 3 is answered as yes for all locations
- time to complete resolution based on the time-point at which all symptoms at all locations have resolved
- therapeutic failure: A patient will be assessed as having had a therapeutic failure if any of the following occurs:
  - time to the beginning of relief of symptoms for the attack based on the overall VAS occurs later than 4 hours after baseline
  - within 4 hours after beginning of treatment administration and after beginning of relief the overall VAS increases again to a value of at least the baseline score
  - the patient has occurrence of HAE at a new location within 4 hours after achieving beginning of relief of symptoms
  - the patient taking any of the medications that may interfere with the assessment of the impact of Ruconest on efficacy measures, between the onset of the attack and prior to time of initial relief of symptoms including:
    - Narcotics
    - plasma-derived C1 inhibitor
    - Fresh frozen plasma
    - Bradykinin receptor antagonist
    - Analgesics
    - Anti-emetics
    - Non Steroidal Anti-Inflammatory Drugs (NSAIDs)

    Any change in current and optional maintenance therapy (androgens and/or anti-fibrinolytics) from the time of the onset of the current attack is not allowed. The dosage of androgens and anti-fibrinolytics may not be increased earlier than 24 hours after the treatment with study medication.

For a detailed description and definition of used terms, see Appendix F.

10.2 Study procedures

10.2.1 Assessment of safety

This section describes all safety measurements recorded in this study; procedures for eliciting reports of adverse events / inter-current illness are further described in protocol section 11.1.

10.2.1.1 General safety measurements

Vital signs including blood pressure (supine systolic and diastolic), pulse and body temperature), and ECG will be considered as general safety parameters.

10.2.1.2 Safety laboratory measurements

Hematology and biochemistry

Samples for routine hematology and biochemistry assays will be collected prior to each infusion and at Day 28.The routine hematology and biochemistry assays will be performed by the clinical centre's local laboratory.

The samples will be analyzed for the following hematolgy parameters: ESR, hemoglobin, hematocrit, red blood cell count (RBC), MCV, MCH, MCHC, total white blood cell count (WBC), platelet count, differential WBC units, neutrophils, lymphocytes, monocytes, eosinophils and basophils.

The samples will be analyzed for the following biochemistry parameters: sodium, potassium, chloride, calcium, inorganic phosphate, total protein, albumin, glucose, creatinine, AST, ALT, LDH, blood urea nitrogen, bilirubin (total), alkalinephosphatase, Gamma-GT, uric acid, total cholesterol, HDL cholesterol, Triglycerides, CRP and amylase.

Pregnancy testing

For female patients capable of bearing children (i.e. postmenarcheal girls), a urinary pregnancy testing for hCG will be performed prior to each treatment with Ruconest. Alternatively, serum hCG may also be performed to test for pregnancy as mandated by the clinical centre and/or Ethics Committee or when urine is not produced.

10.2.1.3 Diagnostic tests, PK/PD and immunological analyses

Following collection, the samples will be kept and processed **at room temperature**. The eventual plasma aliquots should be immediately stored upright at or below -20 °C until shipment to QPS Netherlands BV, where sample analysis will be performed. Investigator manuals will be provided to each center detailing the procedures related to central laboratory sample handling and (dry ice) shipment.

Diagnostic assays

Blood samples for diagnostic assays will be collected at screening. The samples will be analyzed for the following parameters: C1INH activity, C1q, C4 and C1INH auto antibodies (IgM, and IgG anti-rhC1INH); C4 will also be analyzed at baseline of each attack.

PK/PD assays

Blood samples for PK assays will be collected prior to the first treatment, one blood sample directly following infusion (named T 5 min, from a vein in the contra-lateral arm) and another blood sample between 2-4 hours post-infusion. This sampling time points are considered the most useful schedule derived from modelling of adult data. The samples will be analyzed for C1INH activity and C4.

Immunogenicity

The presence of anti-C1INH IgG and IgM antibodies (tested against immobilized rhC1INH), and of antibodies against anti-HRI will be assessed. Cut-off values of each assay were previously determined using plasma samples from normal donors and hereditary angioedema patients not previously exposed to rhC1INH. Plasma samples found to have an antibody test result above the cut-off level of the assay will be tested in confirmatory assay(s):

- Plasma samples above cut-off for anti-C1INH antibody will be tested in an anti-C1INH displacement assay, to determine whether or not increased values observed in the ELISAs

were due to specific or non-specific responses. Samples that are confirmed positive in the displacement assay will then be tested in an assay for neutralizing antibodies.

- Plasma samples that show a response above the cut-off level in the initial anti-HRI ELISA will be tested in a displacement assay.

These tests will be performed on samples obtained at screening, prior to each treatment with rhC1INH, and at the Day 28 and Day 90 follow up visits. If the new attack is treated before completing the follow-up visits for the previous attack, the D28 or D90 visit schedule will be reset based on the most recent treatment.

These tests and antibody assays mentioned above will be performed by QPS Netherlands B.V.

- C1INH activity will be determined as inhibitory activity against C1 esterase with a chromogenic assay.
- C1q assays will be performed by nephelometry. This assay will be performed at screening to confirm the diagnosis of HAE and/or to exclude AAE.
- Levels of C4 antigen will be determined by nephelometry. This will be performed at screening and at baseline of each attack to support the diagnosis of HAE.
- Anti-rabbit epithelium (dander) and anti-cow milk IgE assays will be performed using a validated, commercially available system[8] (ImmunoCAP®, Phadia, Sweden; or equivalent. Prior to treatment with Ruconest a negative test for anti-rabbit epithelium (dander) (cut off <0.35 kU/L) should be documented. Anti-cow milk IgE will be tested once at screening. Anti-rabbit epithelium (dander) IgE will be performed at screening, at the Day 28 follow-up visit of each attack, every year following screening, and if adverse events are observed indicative of hypersensitivity reactions.

10.2.2 Blood sampling procedures and parameters

It is recommended, for the sake of patient's comfort, to use butterfly needles and to place a catheter during the time of hospitalization for easy blood collection. Also, applying a topical anesthetic before catheter placement may be considered.

For central laboratory testing, the amount of blood taken per patient has been carefully considered, to reduce the burden as much as possible. For diagnostic testing, PK/PD analyses, and immunology testing, depending on the need, either 1.8, 2.7 or 4.5 mL of venous blood will be collected at the designated time points (see below and Appendix A), i.e. by using the smallest citrate-tube available. The routine laboratory safety parameters will be assessed in the local laboratories, and the required blood volume may vary between laboratories. At the set-up of the study, special attention will be given to minimize the required blood volume for this routine safety testing. The estimated volumes below are considered the maximally required sample volumes based on 5 mL collection tubes.

The typical amount of blood taken from a patient including a screening visit and following one HAE attack during the 90-day follow-up period will be a maximal volume of 30 mL according to the schedule below (see also appendix A). If subsequent attacks will be treated with the study medication, where PK/PD sampling is no longer scheduled, the volume of blood collection during the treatment period is estimated to account for a maximal volume of 25 mL for each additional attack.

If a 2 year old child was followed up for a single HAE attack (assuming an otherwise healthy child with a weight of 12 kg, and a whole blood volume of 80 mL/kg BW) the total volume of removed blood over a 3 month period would account for 30/960=3.1% of total blood volume. For a 13 year old (assuming an average weight 44 kg and a whole blood volume of 75 ml/kg BW) this would be

under 1%. It is generally accepted that blood loss should not exceed 3% of total blood volume over four weeks, and it should not exceed 1% of total blood volume at any single time[9]. In case of doubt, the investigator should ascertain that the hematocrit is not less than 38 volume%[10].

Blood sampling volumes	Screening	First Attack	Subsequent attacks
Screening diagnostics and immunology	4.5 mL		
Day of attack (immunology and PK/PD (only at first attack))	-	6.3 mL	1.8 mL
Follow up visit at Day 28 (immunology)	-	1.8 mL	1.8 mL
Follow up visit at Day 90 (immunology)	-	1.8 mL	1.8 mL
Laboratory safety parameters (hematology/ biochemistry) at baseline and Day 28 (4 x 5 mL)	-	20 mL[1]	20 mL[1]
Total	**4.5 mL**	**29.9 mL**	**25.4 mL**

(1) Expected maximal volumes: hematology and biochemistry parameters are assessed by the local laboratories: the actual volumes may depend on the local laboratories' requirements and possibilities to minimize sample volume.

Investigator review and sign off

Reports of all safety laboratory results (hematology, biochemistry, immunogenicity) will be reviewed, signed and dated by the investigator. The screening lab results must be reviewed and signed by the investigator to confirm eligibility prior to the administration of study medication.

10.2.3 Assessment of efficacy

The assessment of efficacy will be based on the patient's and the investigator's assessment of angioedema signs and symptoms before administration of rhC1INH and the follow-up of changes in angioedema signs and symptoms at the symptomatic anatomical locations.

The severity of angioedema signs before and after the administration of rhC1INH will be rated by the patient or parent/legal guardian using VAS-scales. VAS completion by the patients is preferred. If needed, depending on the patient's level of understanding, parents may assist in completion. On the VAS-scales, it will be indicated if the score is completed by the patient or parent/legal guardian. Separate VAS forms will be given to express the current feelings considering the severity of angioedema symptoms for five possible anatomical locations: abdominal, urogenital, oro-pharyngeal-laryngeal (OPL), facial, and peripheral locations. The patient or parent/legal guardian will complete the form by placing vertical marks on each of the 100 mm horizontal lines provided to answer each short question related to the severity of symptoms and the patient's condition. Special attention should be given to patient and parent/legal guardian instructions on the use of the VAS prior to an attack (e.g. at screening and at the baseline assessment of each treated attack). Investigators should make sure that the various scales and the low and high end extremes are well understood, by discussing them extensively with patients and their parents/legal guardian.

Each of the VAS instruments provides a patient assessment of the intensity of a range of symptoms pertinent to a symptomatic anatomical location. The Abdominal VAS instrument measures the patient's perceptions relating to four symptoms (illness, pain, bloatedness, and nausea), the OPL VAS instrument measures six symptoms (illness, pain, swelling, breathing, speech, and swallowing), the facial VAS instrument measure three symptoms (illness, pain and swelling), the Peripheral VAS instrument measures three symptoms (swelling, pain, and use of extremity), and the Urogenital VAS instrument measures five symptoms (illness, pain, swelling, nausea, and urination). Additionally, the last question for each attack location is an overall severity VAS for a global assessment of the intensity of the symptoms at that particular location. The wording of the overall severity VAS question is the same for each of the four anatomical location VAS instruments. Examples of the forms are attached as Appendix B.

In addition to completing the VAS forms, patients will answer 3 questions related to the change in severity of their symptoms since the initial assessment. This Treatment Effect Questionnaire (TEQ) will be administered (following confirmation of eligibility) with the VAS for each symptomatic anatomical location. An example of the questionnaire is attached as Appendix C). If needed, depending on the patient's level of understanding, parents may assist in completion.

In case of a new attack location, additional forms (VAS and TEQ) will be provided for the patient/legal guardian to complete and the time will be recorded.

The patient should be observed for 4 hours in the hospital. After each assessment before discharge of the patient from clinical observation, the forms will be stored in the patient's file and will not be available for review by the patient or parent/legal guardian or investigator to preserve non-biased assessment at each time point by both the patient and study physician. The exception to this will be the moment at which the investigator judges the patient's condition well enough for discharge from the hospital, enabling the investigator to verify the patient/parent scores.

The assessment of the angioedema signs by the VAS and TEQ will be performed just before start of infusion, and at T30m, T1h, T2h, T4h, T8h and T24h after study medication infusion as shown in Appendix A.

In a diary, dispensed at discharge from the hospital, the patient will be asked to record the moment at which all angioedema symptoms at all locations have resolved (complete resolution). If needed, depending on the patient's level of understanding, parents may assist in completion.

The investigator will assess the severity of the patient's angioedema symptoms using an IS for each affected anatomical location (see example in Appendix D). In case of a new attack location, additional IS-scores will be recorded. The investigator symptoms score will be assessed at presentation of the acute angioedema attack and at 30m, 1h, 2h, and 4h after study medication infusion as shown in Appendix A. All results of assessments will be documented in the CRF.

10.2.4 Study visit procedures

The sections below list the procedures to be performed for each of the planned time points, as shown in the Schedule of Assessment (see Appendix A).

10.2.4.1 Screening Visit

The following Screening procedures should be performed:

- obtain written informed consent (parental permission) signed by the legal guardian(s)
- record demographics (including gender, race, date of birth)
- general medical history
- perform physical examination (including height and weight), assessment Tanner Stage (see Appendix G)
- record vital signs (supine blood pressure, pulse, body temperature)
- obtain past relevant HAE history and HAE Attack history (including maintenance therapy or prophylaxis of HAE, total number of treatments with C1INH per year, type of attacks, number of attacks per year, impact on Quality of Life and social activities)
- obtain the names of all current prescription and over the counter (OTC) medications, vitamins and supplements including start date, dosage, frequency, route, and indication for usage
- obtain blood sample for the following evaluations by the central laboratory:
    - diagnostic confirmation (C1INH activity, C1q, C4 and C1INH auto antibodies (IgM and IgG anti-rhC1INH))
    - anti-HRI antibodies and IgE antibodies against rabbit epithelium (dander) and cow-milk
- verification of inclusion and exclusion criteria upon availability of all relevant (laboratory) data.

Following the evaluation of a patients' eligibility, the study staff is requested to complete a patient screening form, and to send that (by fax or e-mail) to the CRO (PSR-group B.V) in order to keep them updated on the progress of patients' recruitment.

10.2.4.2 Treatment Visit

An eligible patient may only be treated if the patient presents with an eligible attack. The patients will remain in hospital for 4-5 hours during a treatment visit. The procedures performed at subsequent treatment visits will be the same.

Permitted deviations of actual sampling and assessment time points from those projected in the Schedule of Assessments will be within ten percent, calculated from t = 0 (start of study medication administration) to the relevant activity. The following procedures will be performed over a 4-5 hour period as follows:

At presentation of the attack:

- assessment of current angioedema signs and evaluation of evolution since onset of symptoms, including investigator's symptom score
- perform physical examination (including weight), assessment Tanner Stage (see Appendix G)
- record vital signs (supine blood pressure, pulse, body temperature)
- assess and record changes or additions in current prescription and OTC medications, vitamins and supplements including start date, dosage, frequency, route, indication for usage since the last study visit
- assess and record if any medications previously reported as taken on an as needed basis (PRN) were taken since the onset of symptoms
- record and assess new AE/SAE's or changes to ongoing AE/SAE's since the last visit or last infusion, if applicable
- evaluate all inclusion and exclusion criteria
- 12-lead ECG
- blood sampling for routine laboratory safety parameters, PK/PD (only at first attack) and Immunological Analysis (C1INH auto antibodies (IgM and IgG anti-rhC1INH and anti-HRI) and C4.
- pregnancy test (only for post-menarcheal female patients)
- assessment of baseline angioedema signs by VAS-scales and TEQ just before infusion.

Infusion (T=0h)

- perform infusion of Ruconest

Directly following infusion (T5 min)

- blood sampling for PK (from a vein in the contra-lateral arm)

30 minutes (± 3 min), and 1 hour (± 6 min), 2 hours (± 12 min) and 4 hours (± 24 min) post-Infusion

- assessment of current angioedema signs (patient's VAS, TEQ and IS-score)
- record vital signs (supine blood pressure, pulse, body temperature)
- 12 lead ECG between 30 min and 2 hours
- blood sampling for PK/PD between 2 and 4 hours post-infusion
- record and assess new AE/SAE's or changes to ongoing AE/SAE's since infusion
- record changes in concomitant medication and/or need for rescue medication

The patient should be observed for 4 hours in the hospital. After 4 hours, the investigator may decide to discharge the patient from the hospital if the patient's condition is judged well enough. The investigator may consult the VAS-scores to verify if Time to Beginning of Relief has been

achieved. If the patient's condition is not well enough, the patient will remain in the hospital under the appropriate medical care.

At hospital discharge

- perform physical examination
- Dispensation of diary, in which patients will be instructed to record any changes in their health condition and concomitant treatments following the study medication administration. Also, the time at which there is complete resolution of the angioedema attack symptoms has to be indicated
- Dispensation of additional VAS and TEQ questionnaires. The patients will be instructed to continue completing the VAS-score and TEQ at T8h (± 1 hour) and T24h (± 4 hour) (i.e. at the time of the phone call). The 8 hour time point may be facilitated by a study coordinators phone call.

The diary and questionnaires (VAS and TEQ) are to be returned on the visit at Day 28. If patients report, or if legal guardians notice, deterioration of HAE symptoms, the investigator should be contacted immediately.

10.2.4.3 Follow-up Visits

At 24 hours (± 4h) following treatment administration, a phone call is made to the patient in order to:

- assess HAE symptoms (registering localization, severity and time course) in order to document the further evolution of the acute attack (resolution, exacerbation, late relapse etc), including patient's VAS score and TEQ
- remind about detailed completion of the diary for AEs, concomitant medication
- ask patient if complete resolution of symptoms has occurred, and if so record the exact date and time in the CRF
- record and assess new AE/SAE's or changes to ongoing AE/SAE's since the last infusion
- record changes in concomitant medication and/or need for rescue medication

On Day 28 (± 3 days) following treatment administration, a center visit is scheduled, at which the following procedures are scheduled:

- return of the diary including recording of time of complete resolution of symptoms
- perform routine physical examination
- blood sampling for Immunological Analysis and routine laboratory safety parameters
- assess HAE history during preceding 28 days (e.g. occurrence of intercurrent HAE-episodes etc)
- evaluate diary and record and assess new AE/SAE's or changes to ongoing AE/SAE's since the last infusion

- evaluate diary and record changes in concomitant medication and/or need for rescue medication

On Day 90 (± 7 days) following treatment administration, a center visit is scheduled, at which the following procedures are scheduled:

- perform routine physical examination
- record vital signs (supine blood pressure, pulse, body temperature)
- blood sampling for Immunological Analysis
- HAE history since previous treatment (e.g. occurrence of intercurrent HAE-episodes etc).
- record and assess new AE/SAE's or changes to ongoing AE/SAE's since the last infusion
- record changes in concomitant medication and/or need for rescue medication

Day 28 and Day 90 visits will occur following an occasion treated with Ruconest. If a new attack is treated before completing the follow-up visits for the previous attack, the D28 or D90 visit schedule will be reset based on the most recent treatment. In that case, the diary is to be returned and evaluated at the time the patient presents in the clinic with the new attack.

Independent of the occurrence of eligible acute HAE attacks, a center visit is scheduled at every year (± 1 month) after screening at which the following procedures are scheduled:

- blood sampling for IgE antibodies against rabbit epithelium
- HAE history since previous visit, if applicable (e.g. occurrence of intercurrent HAE-episodes etc)
- record and assess new AE/SAE's or changes to ongoing AE/SAE's since the last visit, if applicable
- record changes in concomitant medication

10.3 Withdrawal of individual patients

Patients can withdraw from the study at any time for any reason without any consequences. The responsible investigator can also discontinue study treatment if continuing such treatment is in his/her opinion deleterious for the patient's well-being or for any other reason.

10.4 Replacement of individual patients after withdrawal

There will be no replacement of patients after withdrawal.

10.5 Follow-up of patients withdrawn from treatment

When a patient withdraws from the study a full medical examination (including blood sampling for routine safety and immunology analyses) will be performed and follow-up visits Day 28 and Day 90 will be completed whenever possible. In case of treatment discontinuation because of severe or serious adverse events additional blood sampling for hematological, blood chemistry and urine laboratory tests or other special examinations may be performed. As described, such patients will continue to be followed as specified by the protocol unless they refuse such follow-up. Patients

who are withdrawn from treatment due to an AE will be monitored closely until resolution of the event or stabilization, at the discretion of the investigator.

10.6 Subject and Study Stopping Rules

Subject stopping rule

A subject who experiences a treatment-emergent anaphylactic/anaphylactoid reaction or a confirmed adverse event of special interest, as specified in section 11.2. will be withdrawn and the case will be reviewed, discussed with the treating investigator, and further evaluated as required.

Study stopping rule

Further enrollment into the study, and further Ruconest administration in all subjects, will be temporarily halted pending a review of the data by the Data Safety Committee if two patients experience a treatment-emergent anaphylactic/anaphylactoid reaction or a confirmed adverse event of special interest, as specified in section 11.2. Only after review of the relevant data by this committee, the study may be reinitiated.

10.7 Premature termination of the study

Both the investigator and the Sponsor reserve the right to terminate the study at any time. Should this be necessary, the procedures will be arranged on an individual study center after review and consultation by both parties. In terminating the study, the Sponsor and the investigator will ensure that adequate consideration is given to the protection of the patients' interests. The pertaining Institutional Review Boards and authorities will be informed of any decision to halt, abandon or continue the study.

10.8 End of study

The end of the study for a given center is defined as the date at which the last patient follow-up in that particular center occurs. The end of study in a given country is defined as the date at which the last patient follow-up in that particular country occurs. The global end of the study is defined as the date at which the last patient follow-up occurs in the last active center.

11 SAFETY REPORTING

11.1 Definition and Handling of Adverse and Serious Adverse Events

11.1.1 Adverse Events

An AE is any undesirable physical, psychological or behavioral effect experienced by a patient during participation in an investigational study, in conjunction with the use of the drug or biologic, whether or not product-related. This includes any untoward signs or symptoms experienced by the patient from the time of signing of the informed consent until completion of the study.

AEs may include, but are not limited to:

- Subjective or objective symptoms spontaneously offered by the patient
- and/or observed by the Investigator or medical staff
- Findings at physical examinations
- Laboratory abnormalities of clinical significance

Disease signs, symptoms, and/or laboratory abnormalities already existing prior to the use of Ruconest are not considered AEs unless they reoccur after the patient has recovered from the preexisting condition or in the opinion of the Investigator they represent a clinically significant exacerbation in intensity or frequency.

Clinical significance is defined as any variation in signs, symptoms, or testing that has medical relevance and may result in an alteration in medical care. The Investigator will continue to monitor the patient until the assessment returns to baseline or until the Investigator determines that follow-up is no longer medically necessary.

The Investigator is responsible for monitoring the safety of patients who have enrolled in the study and for accurately documenting and reporting information as described in this section.

Patients and their legal guardians will be given a diary at discharge from the hospital to record any AE following administration of the study medication until follow-up visit Day 28. Also, they will be instructed to report to the Investigator any AE that is experienced by the patient.

Investigators will pose non-leading questions about the occurrence of AEs at each visit. Investigators are required to document all AEs occurring during the clinical study, commencing with the signing of the ICF through the last follow-up visit (Day 90 after the last treatment).

All AEs occurring following the signature of the ICF but prior to the first dose of Ruconest will be recorded.

All AEs (including AEs described in the patient's diary) will be recorded on designated CRF pages. Each AE is to be characterized (i.e., verbatim term) and information provided regarding its seriousness, start and stop dates, intensity, outcome, and causal relationship with Ruconest.

It is important that investigators record accurate AE terms on CRFs. Wherever possible, a specific disease or syndrome rather than individual associated signs and symptoms will be identified by the Investigator and recorded on the CRF. However, if an observed or reported sign or symptom is not considered a component of a specific disease or syndrome by the Investigator, or is atypical, it should be recorded as a separate AE on the CRF.

HAE attacks (not treated with study medication) will be recorded in a separate section of the CRF.

11.1.2 Adverse Event Intensity

The Investigator will be required to assess the intensity of the adverse drug/biologic experience using the following categories to quantify intensity.

- **Mild:** events require minimal or no treatment and do not interfere with the patient's daily activities.
- **Moderate:** events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe:** events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- **Life threatening**: Potentially life-threatening or disabling. High-risk medical interventions.
- **Fatal:** patient died

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

11.1.3 Adverse Event Relatedness

The Investigator will make a judgment regarding whether or not, in his/her opinion; the AE was related to Ruconest. The Investigator will also evaluate any changes in laboratory values; make a determination as to whether the change is clinically important, and whether or not the changes were related to study medication. However, even if the Investigator feels there is no relationship to the study medication, the AE or clinically significant laboratory abnormality MUST be recorded on the CRF.

Below are guidelines for relationship assessment:

- **Unrelated:** There was no relationship of the adverse experience to the use of the drug or biologic. This may include, but is not limited, to the adverse experience being an expected outcome of a previously existing or concurrent disease, concomitant medication or procedure the patient experienced during their treatment period.
- **Possible:** There was no clear relationship of the adverse experience to the use of the drug or biologic; however, one cannot definitively conclude that there was no relationship.
- **Probable:** While a clear relationship to the drug or biologic cannot be established, the experience is associated with an expected adverse experience or there is no other medical condition or intervention which would explain the occurrence of such an experience.
- **Definite**: The relationship of the use of the drug or biologic to the experience is considered definitively established.

If a causal relationship is considered probable, possible, or definite by the Investigator, the AE is considered to be "related" for purposes of regulatory reporting.

11.1.4 Serious Adverse Events

Serious adverse events (SAEs) will be reportable from the time the patient signs the ICF through the last follow-up visit (Day 90 after the last treatment. Adverse events and serious adverse

events will not be collected in patients who sign the informed consent but are considered a screen failure. Investigators need to complete the Screen Failure CRF which will identify the reason for the screen failure. Patients terminated prematurely from the study due to withdrawal of consent will continue to be followed as specified by the protocol unless they refuse such follow-up. Patients who are withdrawn from treatment due to an adverse event will be monitored closely until resolution of the event or stabilization, at the discretion of the investigator.

An SAE is any AE that results in any of the following outcomes:

- Death
- Life-threatening experience
- Required or prolonged inpatient hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly
- Important medical events that, based upon appropriate medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent 1 of the outcomes listed above.

Life-threatening experience. Any adverse experience that places the patient, in the view of the reporter, at immediate risk of death from the adverse experience as it occurred, i.e., does not include an adverse experience that had it occurred in a more severe form, might have caused death.

Required or prolonged inpatient hospitalization. The adverse experience resulted in an initial inpatient hospitalization or prolonged an existing hospitalization of the patient. If a patient is hospitalized as part of the clinical use of the product, a period of normal hospitalization will be outlined in the protocol or by the judgment of the Investigator. Hospitalizations longer than this period will be prolonged hospitalizations.

Persistent or significant disability/incapacity. An adverse experience that resulted in a substantial disruption of a person's ability to conduct normal life functions.

Congenital Anomaly. The exposure of the patient to the drug or biologic during pregnancy that is judged to have resulted in the congenital anomaly/birth defect.

Important medical events. Adverse experiences that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, may jeopardize the patient or patient and may require medical or surgical intervention to prevent one of the outcomes listed above. Important medical events or interventions may be considered an SAE based upon medical judgment of the Investigator.

11.1.5 Reporting Serious Adverse Events

The necessity and time requirements for reporting of SAEs to Pharming Pharmacovigilance or designee and/or regulatory agencies are as follows:

All SAEs will be reported within 24 hours of the Investigator's first knowledge of the event, even if the experience does not appear to be related to the study medication. The contact details are:

Address: VIGILEX B.V.
Archimedesweg 17
2333 CM Leiden
The Netherlands

Email: safety@vigilex.com
Fax: **+31 (0) 71 524 4001**

- All SAEs will include a detailed description of the event(s). Copies of relevant patient records, autopsy reports, and other documents may be requested by and will be sent to Pharming Pharmacovigilance Department (contact details as above).
- The Institutional Review Boards (IRB) must be notified in writing of any expedited SAEs. All unexpected SAEs associated with the use of the study treatment will be immediately reported to appropriate regulatory agencies by Pharming.
- All AEs and SAEs will be noted on the CRF, with a full description including the nature, date and time of onset and resolution, determination of seriousness, intensity, causality, corrective treatment, and outcome.

11.2 Adverse Events of Special Interest

Adverse Events of Special Interest (AEOSI) are AEs that do not meet any criteria for an SAE but are nonetheless of particular interest in the context of this study. AEOSIs will be collected for all patients during the study period. Although these are non-serious events, the Investigator will be asked to provide detailed initial information and follow up to Pharming (see contact details in section 11.1.5) by using Pharming's CRF and SAE Report Form. Pharming Pharmacovigilance will periodically review the AE data including all AEOSIs.

The list of AEOSI with reporting timelines are presented below:

Adverse Events of Special Interest	Reporting timelines to Pharming PV
Type I hypersensitivity reaction due to pre-existing IgE antibodies against rabbit antigens	24 hrs
Type I hypersensitivity reaction due to cross reaction with IgE antibodies against cow milk antigens	24 hrs
Type I hypersensitivity reaction due to the formation of IgE antibodies against rabbit antigens	24 hrs
Type III hypersensitivity reaction due to the formation of antibodies against Ruconest	24 hrs
Induction of acquired angioedema due to the formation of anti-C1 inhibitor antibodies	24 hrs
Thromboembolic complications	24 hrs

11.3 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.

11.4 Pregnancy

11.4.1 Pregnancy Test and Use of Contraception

No studies of rhC1INH have been conducted in pregnant women. To ensure patient safety, female patients capable of bearing children must have a negative pregnancy test prior to study drug administration.

Note that female patients will be considered capable of bearing children if they are postmenarcheal, unless they have had a hysterectomy or bilateral oopherectomy.

Every effort should be made to prevent pregnancy during this study. All patients of reproductive potential involved in the study are required to use an effective method of contraception during this time. If a patient becomes pregnant, she must not receive any study treatment while pregnant.

11.4.2 Pregnancy Notification and Follow Up

Female patients will be instructed to notify the investigator immediately if they discover they are pregnant. The progress of the pregnancy will be followed until its outcome is known (i.e., delivery, elective termination, or spontaneous abortion). If the pregnancy results in the birth of a child, additional follow-up information may be requested. If the pregnancy results in spontaneous abortion or stillbirth, the event should be reported as an SAE.

Specific instructions regarding documentation of pregnancy are provided in the Safety Plan; however, AEs that occur in study patients who become pregnant are reported as described in Section 11.1.

11.5 Data Safety Committee

No formal (independent) Data Safety Monitoring Board will be installed. However, data will be regularly reviewed by an internal committee composed of representatives of Pharmacovigilance, medical management and project management, supplemented with qualified experts, one international HAE-expert, and one pediatric expert with HAE experience. This committee will discuss and analyze safety issues pertaining to the study. Frequency of meetings and responsibilities will be described in a separate study specific manual.

12 STATISTICAL ANALYSIS

12.1 Analysis sets

The following analysis sets will be considered:

Screening Set: All patients screened for the study, who at screening were eligible for treatment.

Safety Set: All patients enrolled in the study, who received at least one study treatment.

Intention to treat (ITT) Set: All patients enrolled in the study who received at least one study treatment and for whom any efficacy data are available.

Per protocol (PP) Set: All patients of the ITT Set who have at least one attack without any major protocol violation. For these patients only the data of attacks without major protocol violation are retained.

The exclusion of patients or data from the analysis sets will be discussed during a pre-analysis meeting that will be held before database lock.

The analysis of key screening data will be performed on the basis of both the Screening Set and the ITT Set. The analysis of the safety data will be performed on the basis of the Safety Set. The analysis of the efficacy data will be performed on the basis of the ITT Set and the PP Set.

Exploratory analyses based on Tanner Stage may be performed.

12.2 Variables

The following data will be considered in the analysis:

Safety

- immunogenicity data
- adverse events
- laboratory data (hematology, biochemistry).
- vital signs
- ECG

Efficacy

- time to beginning of relief on the basis of the overall severity VAS using the first time-point with a decrease of at least 20 mm at any eligible location, with persistence at the next time-point
- time to minimal symptoms assessed by using the overall severity VAS, defined as the first time point at which the overall severity VAS fall below 20 mm for all assessed locations
- time to beginning of relief on the basis of the IS using the first time-point with a decrease of at least one point at any eligible location
- time to minimal symptoms on the basis of the first time-point at which the IS reaches a value of one or less at all locations
- Time to beginning of relief on the basis of Question 1 and 2 of the TEQ, defined as the first time-point at which Question 1 is answered as a little better, better or much better and

Question 2 is answered as yes for the most severe eligible location, with persistence of these responses at the next time point

- Time to minimal symptoms on the basis of Question 3 of the TEQ, defined as the first time-point at which Question 3 is answered as yes for all locations
- time to complete resolution based on the time-point at which all symptoms at all locations have resolved
- therapeutic failure

12.3 Statistical methods

12.3.1 General considerations

A detailed statistical analysis plan (SAP) will be prepared based on the objectives of the protocol. Procedures for accounting for missing, unused or spurious data will also be included therein. This plan will be finalized as early as possible after creation of the database and at the latest before database lock.

The statistical analysis will consist of:

- Descriptive statistics (mean, median, standard deviation, 95% CI on the mean, minimum, maximum, and number of observations) for the quantitative variables, and frequency distributions (frequencies and percentages) for the categorical variables (binary and ordinal variables). An exact 95% CI will be given for key categorical variables.
- Kaplan-Meier analyses of the time to event variables (time to beginning of relief, time to minimal symptoms and time to complete resolution), with estimates of the median and quartiles with 95% confidence interval.

The statistical analysis will be performed using SAS software for WINDOWS, version 9.2 or later.

12.3.2 Screening data

A description will be given of the key screening data on the basis of both the Screening Set and the ITT Set.

12.3.3 Safety data

The analysis of safety will be performed on the basis of the Safety Set. It will be descriptive and consist of:

<u>Immunogenicity data</u> (anti-C1INH antibodies of IgG and IgM, anti-HRI)

- descriptive statistics at each assessment time (attack/day)
- documented list of all immunology data (including the data of the confirmatory assays), for the patients for whom any value was observed above the cut-off level

Adverse events

AEs will be recorded throughout the study. These will be coded using the MedDRA dictionary, version 13.1 or later. The analysis will be based on the treatment-emergent adverse events (TEAE), which are the AEs with an onset at any time between the start of treatment and 97 days after treatment for any treated attack.

The following documented lists of individual data will be provided for all attacks together:

- documented list of individual data concerning TEAEs
- documented list of baseline adverse events with onset before start of treatment administration for the first attack, and for subsequent, AEs with onset between 98 days since the previous treated attack and the start of treatment administration

The analysis of TEAEs will be performed by attack number and all attacks together. It will consist of:

- frequency distribution of patients with TEAEs, with serious TEAEs, and with TEAEs causing premature discontinuation
- frequency distribution of patients with TEAEs by system organ class
- frequency distribution of patients with TEAEs by system organ class and preferred term
- frequency distribution of patients with related TEAEs by system organ class and preferred term

If relevant:

- frequency distribution of patients with TEAEs by system organ class, preferred term and maximal severity
- frequency distribution of patients with TEAEs by system organ class, preferred term and strongest relationship

HAE attacks

HAE attacks will be separately recorded. The analysis will consist of:

- documented list of individual data concerning HAE attacks

Laboratory data (hematology, biochemistry)

- Descriptive statistics at each assessment time (attack/day).
- For each attack number descriptive statistics of the difference between the recording before treatment and Day 28 (if this visit occurred before the next treatment).
- Frequency distribution of the status (below, within, and above the normal range), at each assessment time.
- For each attack number shift table of the status, between the recording before treatment and Day 28 (if this visit occurred before the next treatment).

Vital signs

- descriptive statistics at each assessment time (attack/day)

ECG

- descriptive statistics at each assessment time (attack/day)
- For each attack number descriptive statistics of the difference between the recording before treatment and after treatment (between 30 min and 2 hours post administration)

12.3.4 Efficacy data

The following analyses will be performed on the basis of the ITT Set and the PP Set, separately for each attack number.

- Kaplan-Meier analysis for time to beginning of relief on the basis of the overall VAS
- Kaplan-Meier analysis for time to minimal symptoms on the basis of the overall VAS
- Kaplan-Meier analysis for time to beginning of relief on the basis of TEQ questions 1 and 2
- Kaplan-Meier analysis for time to minimal symptoms on the basis of TEQ question 3
- Kaplan-Meier analysis for time to complete resolution
- Kaplan-Meier analysis for time to beginning of relief on the basis of the IS
- Kaplan-Meier analysis for time to minimal symptoms on the basis of the IS.
- frequency distribution for therapeutic failure
- descriptive data for each anatomical location:
    - descriptive statistics of the overall VAS at each assessment time, and of the difference between baseline and each assessment after baseline
    - frequency distribution of the IS at each assessment time

The Kaplan-Meier analyses for TTBR and TTMS based on the VAS as well as the frequency distribution of the therapeutic failures will be repeated for the first and last attack for the patients having been treated for more than one attack.

13 ETHICAL CONSIDERATIONS

13.1 Regulation statement

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. The study protocol and any subsequent amendment(s) will be submitted to the Institutional Review Boards (IRBs) of the respective centers. The IRB may consult with experts on pediatric ethical, clinical and psychosocial issues. The study will be conducted in compliance with the protocol, GCP regulations and the applicable regulatory requirements. The designers of this study have also taken into account guideline ICH-E11 "Clinical Investigation of Medicinal Products in the Pediatric Population".

The regulatory application or submission for regulatory approval will be made by the Sponsor as required by national law.

13.2 Recruitment, written consent and assent

The investigator will obtain written informed consent (parental permission) for each patient participating in this study, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study, before applying any study-related procedure to a patient. IRBs may require additional written consent using their own locally approved consent form.

2-13 year old patients are legally unable to provide informed consent. Written consent must be obtained from the patient's legal guardian. Age-appropriate material (by using pictures and drawings) will be prepared to present to the patients. The investigator and legal guardian have to sign and date that document to confirm that the patient has been informed as well as possible. Patients who are able to write their first name will be asked to provide their written assent on this document. All consent forms, patients' explanatory material and assent forms must be approved in advance by the IRB accepted by the investigator's institution.

The investigator will explain that the patients are completely free to refuse to enter the study or to withdraw from it at any time, without any consequences for their further care and without need for justification[1]. The investigator will complete and sign the informed consent section of the CRF for each patient enrolled.

If a legal guardian is unable to read, an impartial witness should be present during the entire informed consent discussion. After the informed consent form and any other written information is provided, read, and explained to the patient or their legal guardian, and after oral consent has been obtained, if capable of doing so, the patient or their guardian should sign and date the informed consent form. The witness should also sign and date the consent form. In addition, the patient or their guardian should, if possible, sign an additional form confirming that the materials provided have been read and explained to them. The witness should also sign and date this additional form.

Each patient and their legal guardians will be informed that the patient's medical records may be reviewed during the course of the clinical study or afterwards, including review by government agencies. Each patient and their legal guardians will be informed that the patient's medical data will be included in a database and may be reviewed during the course of the clinical study or afterwards, including review by government agencies. However, only authorized personnel will review these data. Each patient and their legal guardians should be informed that the Investigator

1 It is to note that if the patient wishes to withdraw, but the parents and investigator feel that it is in the best interests of the patient to stay in the study (e.g. in case of an acute life-threatening attack), then they can overrule the patient.

will protect any personal information not related to the study, and that individuals associated with the trial are bound by the same confidentiality obligations as other health care professionals with regard to patient confidentiality.

13.3 Benefits and risks assessment

It is generally acknowledged that pediatric patients should be given medicines which have been appropriately evaluated for their use; as a consequence, drugs aimed to have indication for children must be formally studied before widespread use.

Results of this study will help further evaluating the balance of benefits and risks within the specific age group.

Given the demonstrated efficacy in adults, the disease process being similar in adults and children and taking into account that Ruconest is a replacement therapy; the outcome of the therapy is likely to be comparable. As a consequence, a real and direct benefit is foreseen for the participating children. Therefore, the current study is considered as a clinical investigation with low risk while presenting the prospect of direct clinical benefit to the study participant.

To minimize risk, investigators and site personnel should be properly trained and experienced in studying the pediatric population, including the evaluation and management of potential pediatric adverse events.

An internal safety monitoring committee will be established that will evaluate the safety aspects of the study on an ongoing basis to ensure the safety and welfare of the participating patients.

13.4 Compensation for injury

The chance of injury as a result of the study is small. However, the Sponsor has insured this risk by taking a liability insurance which is in accordance with the legal requirements in diverse countries. This insurance provides cover for damage to research patients through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study. A condition is that the damage has been communicated in writing to the Sponsor and the insurance company within this period.

13.5 Incentives

Only costs associated with study participation will be reimbursed, such as transportation costs, etc.

14 ADMINISTRATIVE ASPECTS AND PUBLICATION

14.1 Access to Source Data and Documents

The investigator will permit study-related monitoring, audit(s), IRB review(s) and regulatory inspection(s), by providing direct access to source data/records. Most of the tests being performed are study-related procedures and would not have been regularly performed if the patient was not in the study.

All study data will be handled confidentially. The investigators and responsible CROs will retain the originals of all source documents generated at the various study centers, local and Central laboratories for a period of 15 years after the report of the study has been finalized, after which all study-related documents can be archived and maintained according to GCP regulations. After 15 years the Sponsor will be notified that the source documents can be retained with the Sponsor or destroyed.

14.2 Quality Control and Quality Assurance

This study will be conducted according to the Sponsor's and CRO's Standard Operating Procedures. Quality assurance will be performed under the responsibility of the Sponsor and CRO QA manager.

14.2.1 Study Center Visits and Periodic Monitoring

Pre-study visits will be performed to ensure that centers are well equipped to perform the study and to evaluate the recruitment potential. At the pre study visit, special attention will be given to the following aspects to ensure that the participants' experiences in the trial are positive and to minimize discomfort and distress, according to ICH-E6(R1) "Guideline for Good Clinical Practice" and ICH-E11 "Clinical Investigation of Medicinal Products in the Pediatric Population" and published guidelines[11].

- personnel knowledgeable and skilled in dealing with the pediatric population and its age-appropriate needs, including skill in performing pediatric procedures
- a physical setting with furniture, play equipment, activities, and food appropriate for age
- the conduct of studies in a familiar environment such as the hospital or clinic where participants normally receive their care

All above aspects will all be evaluated before it is decided to retain the clinical center or not in the study.

Shortly before the launch of the study, a Site Initiation Visit will be performed, during which all protocol modalities will be discussed, including CRF completion. Special attention should be given to safety related aspects and the safety reporting procedures put in place (SAEs and AEOSIs). Also, options to minimize discomfort of procedures will be discussed, such as:

- topical anesthesia to place IV catheters
- indwelling catheters rather than repeated venipunctures for blood sampling

The CRO will appoint study Monitor(s) who will contact and visit the investigator(s) regularly. The above will be continuously monitored and discussed. The study Monitor will be allowed, on request, to inspect the various records of the study. It will be the study Monitor's responsibility to

inspect the CRFs at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, correctness and accuracy of all CRF entries. The study Monitor should have access to laboratory test reports and any other source records and data needed to verify the entries on the CRFs. The investigator agrees to cooperate with the study Monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

After source document verification and review by the study Monitor, the original and a copy of the CRF will be collected by the study Monitor while 1 copy will remain at the investigational site. The original will be sent to the CRO and 1 copy will remain with the study Monitor.

The Sponsor will only consider CRFs to be complete when each CRF has been reviewed and signed by the Investigator, indicating their assurance of the accuracy of all recorded data.

14.2.2 Audit and Inspection

This study will be conducted according written SOPs from the Sponsor and CRO, ICH-GCP guidelines and local applicable laws. Audits and inspections can be conducted by health authorities, the Sponsor, the CRO and/or delegates from these.

The investigator will make source data and documents for this study available to a medically qualified clinical quality assurance auditor after appropriate notification.

Quality control should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

14.3 Confidentiality of Patients' Data

The responsible investigator will ensure that the patient's anonymity will be preserved. On CRFs or any other documents submitted to the Sponsor, the patients will not be identified by their names, but by an identification code, consisting of a patient study number. Documents not for submission to the Sponsor, i.e. the confidential patient identification code, original consent forms and source records will be maintained by the investigator in strict confidence.

14.4 Data handling and Record Keeping

14.4.1 Responsibilities

Monitoring will be done by the CRO, in case this responsibility is delegated in part or totally to a third party, the study protocol must be amended. The CRO will be in charge of data management and analysis of data.

QPS Netherlands B.V. will perform the diagnostic and immunosafety assays.

14.4.2 Information of Investigators

All participating investigators will be informed about the methods for rating relevant study outcome criteria, safety reporting modalities, and how to complete the CRFs. The investigators will be kept informed of important information related to the safe use of the IMP as the study proceeds.

14.4.3 Case Report Forms

For each patient enrolled, a CRF will be completed and signed by the investigator or an authorized sub-Investigator. All forms will be filled out using an indelible pen, and must be legible.

The results of the VAS, TEQ and IS will be recorded directly into the CRF without other source documents other than the statement that the named forms are completed. Any other information in the CRF needs to have source documentation.

14.4.4 Changes to Case Report Form Data

Errors occurring in CRFs will be crossed out without obscuring the original entry, the correction will be written alongside the initial entry, and the change will be initialed and dated by the Investigator or designee. Corrective fluids must not be used. When changes to CRF data are necessary following removal of the original CRF from the study center, any such changes will be documented on data clarification/resolution forms, which will be signed by the investigator.

14.4.5 Investigator Site File

The investigator is responsible for maintaining all records to enable the conduct of the study to be fully documented. Copies of the study protocol, study approval letters, all original informed consent forms, center copies of all CRFs, study medication dispensing and accountability logs, and all correspondence pertaining to the study should be kept by the investigator for the maximum period of time required by local regulations; the default time period is 15 years. All such records will be kept confidentially, and not shown to persons outside of the study team.

The investigator is responsible for maintaining a confidential patient identification code, which provides the unique link between named source records and anonymous CRF data for the Sponsor. The investigator must arrange for the retention of this confidential list for at least fifteen years after the completion or discontinuation of the study.

No study document should be destroyed without prior written agreement between the investigator and the Sponsor. Should the investigator elect to assign the study documents to another party, or move them to another location, the Sponsor must be notified.

14.4.6 Provision of Additional Information

On request, the investigator will supply the Sponsor with additional data relating to the study, or copies of relevant source records, which have been made anonymous.

14.5 Changes in the Conduct of the study

14.5.1 Protocol Amendments

Any prospective change to the protocol will be submitted to the approving IRB(s). A ‘substantial amendment’ is defined as an amendment to the terms of the EC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the patients of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or

- the quality or safety of any intervention used in the trial

All substantial amendments will be submitted for approval to the EC and to the Competent Authority (CA).

Non-substantial amendments will be notified to the EC and to the CA and will be recorded and filed by the Sponsor. Examples of non-substantial amendments are typing errors and administrative changes like changes in names, telephone numbers and other contact details of involved persons mentioned in the submitted study documentation.

14.6 Annual Reporting

The Sponsor/investigator will submit a summary of the progress of the study to the IRB once a year. Information will be provided on the date of inclusion of the first patient, numbers of patients included and numbers of patients that have completed the study, SAEs/ SARs, other problems, and amendments.

The Sponsor will submit, once a year throughout the clinical study, a safety report to the EC/IRB and CAs of the concerned EU Member States. In addition to the expedited reporting of SUSARs, this safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study
- a report concerning the safety of the patients, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation

14.7 Clinical Study Report

The Sponsor will notify the EC/IRB and the CA 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 visit.

If the end of study is defined otherwise, this new definition should be given. In case the study is ended prematurely, the Sponsor will notify the EC/IRB and the CA within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the Sponsor will submit a final clinical study report with the results of the study, including any publications/abstracts of the study, to the IRB and the CA.

14.8 Publication Policy

The results of this study will be published in a peer-reviewed journal if applicable and/or if considered appropriate. Conditions are subject to (a) separate contract(s) between the responsible investigators, the sub investigators and the Sponsor.

15 SUMMARY OF CHANGES FROM PROTOCOL VERSION 09 NOVEMBER 2012 TO PROTOCOL VERSION 10 JUNE 2014

The changes from version 09 November 2012 to version 10 June 2014 are non-substantial.
The contact details of the Sponsor, Contract Research Organisation and Pharmacovigilance service provider have been updated. The time windows for the visits have been added and further detailed guidance is provided on collection of PRN used medications. Definitions for relapse and spontaneous regression were added.

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17 APPENDICES

Appendix A: Schedule of Assessments C1 1209

	Screen	Occasion									Post			every year after screen
Day:		Day 0									Day 1	Day 28	Day 90	
Type of Visit:	*Center Visit*	Center Treatment Visit									*Phone*	*Center Visit*	*Center Visit*	*Center Visit*
Time:		P[2]	0'	5'	30'	1h	2h	4h	at dis-charge[3]	8h[3]	24h	D28	D90	
Study medication infusion[1]			X											
Informed Consent	X													
Demographics	X													
Medical History / HAE-disease history	X													
Evaluation in- and exclusion criteria	X	X												
Physical examination	X	X							X			X	X	
Vital signs (blood pressure, pulse, body T)	X	X			X	X	X	X					X	
ECG		X			----X----									
Pregnancy test[9]		X												
Blood sampling for diagnostic testing[5]	X *(4.5 mL)*													
IgE testing[6]												X		X
Blood sampling for immunology[7]		X *(2.7 mL)*										X	X	
Blood sampling for PK/PD[8]				X			----X----							
Blood sampling for hematology / biochemistry		X *(10 mL)*										X *(10 mL)*		
Clinical assessment of angioedema signs		X									X[4]			
Investigator's symptom score		X			X	X	X	X						
Patient/Parent's VAS + TEQ		X[2]			X	X	X	X		X	X			
Dispensation of diary									X					
Return and evaluation of diary												X		
Intercurrent HAE episode(s)												X	X	X
Assessment of Adverse events		X	----------X----------								X	X	X	X
Concomitant medication	X	X	----------X----------								X	X	X	X
Total blood volume (mL):	4.5	12.7		1.8			1.8					11.8	1.8	1.8

(1) Catheter system to be used if possible, which can be used for blood sampling and treatment administration (and a second dose if necessary), and can be left for 4 hours. In order to minimize patient distress, local, topical anesthesia may be considered before placing a catheter.

(2) P = at presentation with an acute angioedema attack. Assessments and blood samples should be done as close as possible to the time of treatment administration; the VAS and TEQ should be completed immediately prior to study medication infusion.

(3) The patient may be discharged after 4 hours; in that case the patient questionnaire at T8h will not be completed during hospitalization but at home by the patient/legal guardian; the questionnaire is to be returned at the Day 28 visit.

(4) Telephone interview regarding HAE-symptoms in order to document possible therapeutic failure, or late relapse, registering localization, severity and time course.

(5) C1INH activity, C1q, C4, ant bodies against rhC1INH (confirmation + neutralizing antibodies); C4 will be repeated at baseline of each attack.

(6) Anti rabbit epithelium (dander) and anti cow milk IgE testing at screening. Anti rabbit epithelium (dander) IgE only is tested at the Day 28 follow-up visit of each attack and every year after screening.

(7) IgM and IgG ant bodies against C1INH (confirmation + neutralizing) and HRI (+confirmation), and C4 (only at screening and baseline of each attack)

(8) C1INH activity (PK) and C4 (PD), only at first attack, at baseline, directly following infusion and one additional sample within the window of 2-4 hours

(9) If relevant, i.e. only for post-menarcheal female patients

Appendix B: Visual Analogue Scales

VISUAL ANALOGUE SCALES (ABDOMINAL)

These VAS-scores were completed by: ❑ patient

❑ parent/legal guardian

Below, you can see some lines. We would like you to help the doctor to understand how you feel right now, by making a mark on each of those lines. Sick people of all ages use this way to show their doctors how they feel. If you don't understand any of the words below, please ask your Mom or Dad to explain. If you don't want to mark the lines, then your Mom or Dad can mark the lines for you.

How to mark the lines: you will see that at one end of each line, for example for pain (number 2), it says, 'no pain'. At the other end of each line, it says, 'worst pain'. So you can put a mark at one end of the line if you feel really good, or at the other end of the line, if you feel as bad as possible. You can also put a mark somewhere in the middle, to show the doctor just how you feel.

1. How ill do you feel ?

Not at all ill |——————————————————————| Extremely ill

2. Do you have abdominal pain ?

No pain |——————————————————————| Extremely painful

3. Do you feel bloated ?

No, totally empty |——————————————————————| Unbearably full

4. Do you feel nauseous ?

No nausea at all |——————————————————————| Extremely nauseous

5. How severe are the angioedema symptoms **now** for **this location** ?

No symptoms |——————————————————————| Extremely disabling

VISUAL ANALOGUE SCALES (UROGENITAL)

These VAS-scores were completed by: ❑ patient

❑ parent/legal guardian

Below, you can see some lines. We would like you to help the doctor to understand how you feel right now, by making a mark on each of those lines. Sick people of all ages use this way to show their doctors how they feel. If you don't understand any of the words below, please ask your Mom or Dad to explain. If you don't want to mark the lines, then your Mom or Dad can mark the lines for you.
How to mark the lines: you will see that at one end of each line, for example for pain (number 2), it says, 'no pain'. At the other end of each line, it says, 'worst pain'. So you can put a mark at one end of the line if you feel really good, or at the other end of the line, if you feel as bad as possible. You can also put a mark somewhere in the middle, to show the doctor just how you feel.

1. How ill do you feel ?

Not at all ill |———————————————————————| Extremely ill

2. Do you have pain in the genital area ?

No pain |———————————————————————| Extremely painful

3. Do you have any swelling in the genital area ?

No swelling |———————————————————————| Extreme swelling

4. Do you feel nauseous ?

No nausea at all |———————————————————————| Extremely nauseous

5. How easily can you urinate ?

Easily |———————————————————————| Cannot urinate at all

6. How severe are the angioedema symptoms **now** for **this location**?

No symptoms |———————————————————————| Extremely disabling

VISUAL ANALOGUE SCALES (ORO-PHARYNGEAL and/or LARYNGEAL)

These VAS-scores were completed by: ❑ patient

❑ parent/legal guardian

Below, you can see some lines. We would like you to help the doctor to understand how you feel right now, by making a mark on each of those lines. Sick people of all ages use this way to show their doctors how they feel. If you don't understand any of the words below, please ask your Mom or Dad to explain. If you don't want to mark the lines, then your Mom or Dad can mark the lines for you.
How to mark the lines: you will see that at one end of each line, for example for pain (number 2), it says, 'no pain'. At the other end of each line, it says, 'worst pain'. So you can put a mark at one end of the line if you feel really good, or at the other end of the line, if you feel as bad as possible. You can also put a mark somewhere in the middle, to show the doctor just how you feel.

1. How ill do you feel ?

Not at all ill ——— Extremely ill

2. Do you have pain in your mouth or throat ?

No pain ——— Extremely painful

3. Do you have any swelling in your mouth or throat ?

No swelling ——— Extreme swelling

4. How easy is it to breathe ?

No problems ——— Very difficult to breath

5. Is your speech affected ?

Not at all ——— Extremely affected/ Cannot speak at all

6. Do you have difficulty in swallowing ?

Not at all ——— Great difficulty

7. How severe are the angioedema symptoms **now** for **this location** ?

No symptoms ——— Extremely disabling

VISUAL ANALOGUE SCALES (FACIAL)

These VAS-scores were completed by: ❑ patient

❑ parent/legal guardian

Below, you can see some lines. We would like you to help the doctor to understand how you feel right now, by making a mark on each of those lines. Sick people of all ages use this way to show their doctors how they feel. If you don't understand any of the words below, please ask your Mom or Dad to explain. If you don't want to mark the lines, then your Mom or Dad can mark the lines for you.
How to mark the lines: you will see that at one end of each line, for example for pain (number 2), it says, 'no pain'. At the other end of each line, it says, 'worst pain'. So you can put a mark at one end of the line if you feel really good, or at the other end of the line, if you feel as bad as possible. You can also put a mark somewhere in the middle, to show the doctor just how you feel.

1. How ill do you feel ?

Not at all ill |——————————————————————| Extremely ill

2. Do you have pain in your face?

No pain |——————————————————————| Extremely painful

3. Do you have any swelling of your face?

No swelling |——————————————————————| Extreme swelling

4. How severe are the angioedema symptoms **now** for **this location**?

No symptoms |——————————————————————| Extreme disabling

VISUAL ANALOGUE SCALES (PERIPHERAL LOCATIONS)

These VAS-scores were completed by: ❑ patient

❑ parent/legal guardian

Below, you can see some lines. We would like you to help the doctor to understand how you feel right now, by making a mark on each of those lines. Sick people of all ages use this way to show their doctors how they feel. If you don't understand any of the words below, please ask your Mom or Dad to explain. If you don't want to mark the lines, then your Mom or Dad can mark the lines for you.
How to mark the lines: you will see that at one end of each line, for example for pain (number 2), it says, 'no pain'. At the other end of each line, it says, 'worst pain'. So you can put a mark at one end of the line if you feel really good, or at the other end of the line, if you feel as bad as possible. You can also put a mark somewhere in the middle, to show the doctor just how you feel.

1. Do you have any swelling ?

No swelling |_____| Extreme swelling

2. Do you have pain in your extremities ?

No pain |_____| Extremely painful

3. Do you have difficulty using the swollen extremity ?

No problems |_____| Extremely difficult
Can easily move

4. How severe are the angioedema symptoms **now** for **this location**?

No symptoms |_____| Extremely disabling

Appendix C: Treatment Effect Questionnaire

Treatment Effect Questionnaire Pre-dose

Below, you see 3 questions. We would like you to help the doctor to understand if you feel better or worse since you arrived in the hospital. Sick people of all ages use this way to show their doctors how they feel. Some sentences and words may be difficult for you to understand. You can ask your Mom or Dad to help you.

Question 1: To what extent has the overall severity of your [*abdominal*[1]] HAE attack changed since your arrival at the study center?

Much worse	Worse	A little worse	Not changed	A little better	Better	Much better
□	□	□	□	□	□	□

Question 2: Overall, has the intensity of your [*abdominal*[1]] HAE attack symptoms begun to decrease noticeably since your arrival at the study center?

□ Yes □ No

Question 3: At this moment, are your [*abdominal*[1]] HAE attack symptoms minimal (barely noticeable)?

□ Yes □ No

[1] abdominal is replaced by the relevant attack location

Treatment Effect Questionnaire after Treatment

Below, you see 3 questions. We would like you to help the doctor to understand if you feel better or worse since you got the infusion. Sick people of all ages use this way to show their doctors how they feel. Some sentences and words may be difficult for you to understand. You can ask your Mom or Dad to help you.

Question 1: To what extent has the overall severity of your [*abdominal*[1]] HAE attack changed since you received the infusion?

Much worse	Worse	A little worse	Not changed	A little better	Better	Much better
□	□	□	□	□	□	□

Question 2: Overall, has the intensity of your [*abdominal*[1]] HAE attack symptoms begun to decrease noticeably since your received the infusion?

□ Yes □ No

Question 3: At this moment, are your [*abdominal*[1]] HAE attack symptoms minimal (barely noticeable)?

□ Yes □ No

[1] abdominal is replaced by the relevant attack location

Appendix D: Investigator Score

Please use the following severity score for the evaluation of the HAE symptoms:

Severity score	Evaluation					
0	No symptoms					
1	Almost no symptoms					
2	Mild symptoms					
3	Moderate symptoms					
4	Severe symptoms					
5	Life-threatening					
Abdominal	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Urogenital	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Oro-Pharyngeal / Laryngeal	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Facial	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Peripheral	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
If peripheral, please specify the location(s) below:						
Peripheral:						

Appendix E: Pharmaceutical instructions

Background

Recombinant human C1INH is a powder for solution for injection that must be dissolved with water for injections: it is supplied in a 25 ml colorless type I glass vial. The closure consists of a siliconized chlorobutyl rubber stopper and a flip-off seal of aluminum and white-colored plastic.

Each vial of Ruconest is for single use only.

Aseptic technique should be used for reconstitution, combining and mixing the solutions.

Reconstitution

Reconstitute each vial of Ruconest (2100 U rhC1INH) with 14 ml water for injections (WFI). Add sterile WFI slowly to avoid forceful impact on the powder and mix gently to avoid foaming of the solution. The reconstituted solution contains 150 U/ml rhC1INH and appears as a clear colorless solution.

Visually inspect the reconstituted solution in each vial for particulate matter and discoloration. Do not use solution exhibiting particulates or discoloration. The product should be used immediately.

Dose calculation

The recommended dose of Ruconest is 50 U/kg body weight for patients up to 84 kg; and 4200U (2 vials) for patient from 84 kg or greater.

Determine the patient's bodyweight and calculate the **total volume** of the reconstituted solution **required** to be administered to the patient using the following equation:

$$\text{Volume to be administered (ml)} = \frac{\text{body weight (kg) times 50 (U/kg)}}{\text{150 (U/ml)}} = \frac{\text{body weight (kg)}}{3}$$

Reconstitute **each vial** with 14 ml WFI. Withdraw the required volume from the vials, making sure that the withdrawn volume from the required number of vials is mixed gently. Round up every mL to the next integer number. (e.g., if total required volume is 8.33 mL (for a child weighing 25 kg), withdraw 9 mL from the reconstituted volume).

Discard the vials containing the reconstituted solution not used during this procedure.

Appendix F: List of Definitions

Abdominal attack:
Patients showing symptoms related to swelling of submucosal tissue of the gastrointestinal tract. If this diagnosis of an abdominal HAE attack is not obvious or questionable, appropriate diagnostic efforts including but not limited to abdominal/pelvic ultrasound, should be undertaken to rule out a potential acute abdomen (acute abdominal emergencies).

Angioedema:
The recurrent swelling (increased amount of interstitial fluid in subcutaneous and/or submucosal tissue), occurring in patients with hereditary or acquired angioedema, related to deficiency of functional C1INH.

Eligible attack:
An acute attack of angioedema in the abdomen, facial-oro-pharyngeal region, larynx, urogenital region and/or at peripheral localizations, with onset of eligible symptoms within 5 hrs or less from the time of determination of eligibility by the study physician, in a patient with HAE, who has a level of functional C1INH of less than 50 % of that in pooled normal plasma. An HAE attack in a patient with HAE will be considered eligible if the IS score for at least one anatomical location at the time of initial evaluation is at least 3.

Eligible site/location:
Angioedema at site(s) with onset of less than 5 hrs ago and if the IS score at this location at initial evaluation is at least 3.

Eligible symptoms:
Symptoms of angioedema in the abdomen, facial-oro-pharyngeal region, larynx, urogenital region, and/or at peripheral localizations (subcutaneous edema; most often at the extremities); including but not limited to visible swelling and/or voice change, speech problems, stridor, dyspnea, urine passage problems, pain, distress, anxiety, nausea, vomiting, diarrhea, fainting, dehydration, swallowing problems/dysphagia, hypotension, hypovolemia, tachycardia, ascites.

Exacerbation:
Worsening or increase of angioedema, and/or associated symptoms, and/or involvement of additional anatomical locations.

Laryngeal edema:
Clinical signs of laryngeal edema include dysphagia, the sensation of a lump in the throat, a feeling of tightness in the throat, voice changes, including hoarseness and roughness, stridor, and dyspnea. Additional signs of progressed laryngeal edema may include fear of asphyxiation, and aphonia[12].

New site(s), new manifestation(s), new location(s):
Acute angioedema attack at a new location will be identified as an IS score greater than 0 at any time up to (and including) 24 hours for a location that had an IS score of 0 (or missing IS score) at Baseline.

Peripheral edema:

Angioedema at peripheral localizations (occurring in subcutaneous rather than submucosal tissue), i.e. not localized in the abdomen (gastrointestinal tract) and/or in the facial-oro-pharyngeal region and/or in the urogenital region ('submucosal' sites), e.g. swelling at extremities, hand, wrist, lower arm, upper arm, foot, leg, ear, chest wall, abdominal wall, neck, buttocks.

Early relapse:
Beginning of relief of symptoms by overall VAS within four hours, followed by an increase of VAS score to baseline value or higher, within the first 8 hours after baseline.

Late relapse:
Beginning of relief of symptoms by overall VAS within four hours, followed by an increase of VAS value to baseline value or higher, more than 8 hours after baseline and at most 24 hours after baseline.

Spontaneous regression:
Decrease in symptoms of angioedema scored with investigator score at t = 0 h compared with score at the time of initial evaluation by 1 or more at an eligible location.

Submucosal attack:
Attack with presumed or visible involvement of submucosal tissue at any of the possible eligible sites, i.e. localized in the abdomen (gastrointestinal tract), facial-oro-pharyngeal region, larynx, and/or urogenital region.

Therapeutic failure:
Time to the beginning of relief based on the VAS later than 4 hrs after study medication administration, relapse of the attack within 4 hrs, and/or occurrence of HAE at a new location within 4 hours after achieving beginning of relief of symptoms. The episode will also be considered a failure if the patient recorded taking medications that may have interfered with the assessment of the impact of Ruconest on efficacy measures, prior to time of initial relief of symptoms including: narcotics, plasma-derived C1 inhibitor, recombinant human C1 inhibitor, fresh frozen plasma (FFP), or anti-emetics.

Time to beginning of relief:

Time to the beginning of relief based on patient's VAS scores; time lapsed from the beginning of the infusion of study medication to the beginning of an effect. This variable will be based on the overall VAS for each eligible location. Time to beginning of relief is defined as the first time point at which the "overall severity VAS" decreases by at least 20 mm with persistence of the decrease at the next assessment time so that for the next value at the location a decrease of at least 20 mm with respect to baseline is also observed. Time to beginning of relief will also be calculated based on the IS and Questions 1 and 2 of the TEQ.

Time to minimal symptoms:
Time to minimal symptoms based on patient's VAS scores; time from the start of the infusion of study medication to the first assessment time at which the overall severity VAS reaches a value of less than 20 mm for all locations. Time to minimal symptoms will also be calculated based on the IS and Question 3 of the TEQ.

Time to complete resolution:
Time to complete resolution based on the time, as indicated by the patient in the diary, at which all angioedema symptoms are fully resolved at all locations.

Appendix G: Definitions of Tanner Stages

The Tanner sexual development stages range from stage I to stage V. Below, the definition for the Tanner stages is depicted as extracted from the online Family Practice Notebook website: http://www.fpnotebook.com/Endo/Exam/MlTnrStg.htm

Male Tanner stages	Female Tanner stages
1. Tanner Stage 1 (Prepubertal) 1. Height increases at basal rate: 5-6 cm/y 2. Testes Smaller than 4 ml or long axis <2.5 cm 3. Pubic Hair No coarse, pigmented hair 4. Penis Stage No growth	1. Tanner Stage 1 (Prepubertal) 1. Height increases at basal rate: 5-6 cm/y 2. Breast 1. Papilla elevation only 3. Pubic Hair 1. Villus hair only 2. No coarse, pigmented hair
2. Tanner Stage 2 1. Height increases at basal rate: 5-6 cm/y 2. Testes 1. Size 4 ml or long axis 2.5 to 3.2 cm 2. Age 11.5 years (age 9.5 to 13.5 years) 3. Pubic Hair 1. Minimal coarse, pigmented hair at base of penis 2. Age 12.0 years (age 9.9 to 14.0 years) 4. Penis Stage 1. Earliest increased length and width 2. Age 11.5 years (age 10.5-14.5 years)	2. Tanner Stage 2 1. Height increases at accelerated rate: 7-8 cm/y 2. Breast 1. Breast buds palpable and areolae enlarge 2. Age 10.9 years (8.9-12.9 years) 3. Pubic Hair 1. Minimal coarse, pigmented hair mainly on labia 2. Age 11.2 years (9.0-13.4 years) 4. Modifications based on increasingly earlier Puberty 1. White: Stage 2 changes may appear one year earlier 2. Black: Stage 2 changes may appear two years earlier
3. Tanner Stage 3 1. Height increases at accelerated rate: 7-8 cm/y 2. Testes 1. Size 12 ml or long axis 3.6 cm 2. Age 14.0 years (11.5-16.5 years) 3. Pubic Hair 1. Coarse, dark curly hair spread over the pubis 2. Age 13.1 years (11.2-15.0 years) 4. Penis Stage 1. Increased length and width 2. Age 12.4 years (10.1-14.6 years) 5. Other Changes 1. Gynecomastia may occur (age 13.2 years) 2. Voice breaks (age 13.5 years) 3. Muscle mass increases	3. Tanner Stage 3 1. Height increases at peak rate: 8 cm/y (age 12.5) 2. Breast 1. Elevation of breast contour; areolae enlarge 2. Age 11.9 years (9.9-13.9 years) 3. Pubic Hair 1. Dark, coarse, curly hair spreads over mons pubis 2. Age 11.9 years (9.6-14.1 years) 4. Other changes 1. Axillary hair develops (13.1 years) 2. Acne Vulgaris develops (13.2 years)

Male Tanner stages (cont.)	**Female Tanner stages (cont.)**
4. Tanner Stage 4 1. Height increases at peak rate: 10 cm/y (age 13.8) 2. Pubic Hair 1. Hair of adult quality 2. Not spread to junction of medial thigh with perineum 3. Age 13.9 years (12.0-15.8 years) 3. Penis 1. Continued growth in length and width 2. Age 13.2 years (11.2-15.3 years) 4. Testes 1. Length 4.1 to 4.5 cm 5. Other Changes 1. Axillary hair (age 14.0 years) 2. Voice changes (age 14.1 years) 3. Acne Vulgaris (age 14.3 years)	4. Tanner Stage 4 1. Height increases at 7 cm/y 2. Breast 1. Areolae forms secondary mound on the breast 2. Age: 12.9 years (10.5-15.3 years) 3. Pubic Hair 1. Hair of adult quality 2. No spread to junction of medial thigh with perineum 3. Age: 12.6 years (10.4-14.8 years)
5. Tanner Stage 5 1. No further height increases after age 17 years 2. Pubic Hair 1. Adult pubic hair distribution (15.3 years) 2. Pubic hair spreads to medial thigh 3. No hair spread to linea alba 3. Penis 1. Mature genital size by 16.5 years 4. Testes 1. Length >4.5 cm 5. Secondary sexual characteristics 1. Facial hair present on sides 2. Mature male physique 3. Gynecomastia disappears	5. Tanner Stage 5 1. No further height increases after age 16 years 2. Breast 1. Adult breast contour 2. Areola recesses to general contour of breast 3. Pubic hair 1. Adult distribution of hair 2. Pubic hair spreads to medial thigh 3. Pubic hair does not extend up linea alba
Reference Marshall WA, Tanner JM Variations in the pattern of pubertal changes in boys. .Arch Dis Child. 1970;45(239):13-23	References Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch. Dis. Child. 1969; 44 (235): 291–303