Clinical Study Protocol

Recombinant human C1 esterase inhibitor (rhC1INH) (Ruconest®) in the prevention of severe SARS-CoV-2 infection in hospitalized patients with COVID-19: a randomized, parallel-group, open-label, multi-center pilot trial in the United States (PROTECT-COVID-19-US).

Short title	Prevention of severe SARS-CoV-2 infection in hospitalized patients with COVID-19
Protocol No	C1 6201
Study Phase	2a
Product Name	Ruconest®
Indication	Prevention of severe SARS-CoV 2 with patients with COVID 19
CSP Version	Version 4.0 **Date** 25 May 2021
NCT Number	NCT04530136
Sponsor	Pharming Technologies B.V. Darwinweg 24 2333 CR Leiden, The Netherlands (Pharming Technologies B.V. is a subsidiary of Pharming Group N.V.)
Safety Reporting	Pharming Pharmacovigilance Pharming Healthcare Inc. 10 Independence Blvd, 4th Floor Warren, NJ 07059 Fax: 1 201-389-8092 E-mail: SafetyUS@pharming.com
Medical Monitor(s):	Anurag Relan, MD A.Relan@pharming.com
CRO	Not applicable

Study Type:	Pharming Technologies, B.V. (Sponsor) clinical trial with Investigational Medicinal Product (IMP)
Study Registration:	NCT04530136
Study Identifier:	C1 6201
Sponsor:	Pharming Technologies, B.V. Darwinweg 24 2333 CR Leiden, The Netherlands (Pharming Technologies B.V. is a subsidiary of Pharming Group N.V.)
Principal Investigator:	Jonathan A. Bernstein, MD Professor of Medicine University of Cincinnati College of Medicine Department of Internal Medicine Division of Immunology, Allergy Section 231 Albert Sabin Way ML #563 Cincinnati, Ohio 45267-0563 BERNSTJA@UCMAIL.UC.EDU
Investigational Product:	rhC1INH (Ruconest®)
Protocol Version and Date:	Amendment 3, Version 4.0 Dated 25 May 2021 Amendment 2, Version 3.0 Dated 12 January 2021 Amendment 1, Version 2.0 Dated 09 September 2020 Original Protocol Version 1.0 Dated 08 July 2020

CLINICAL STUDY PROTOCOL SIGNATURE SHEET

Study number C1 6201

Study Title Recombinant human C1 esterase inhibitor (rhC1INH) (Ruconest®) in the prevention of severe SARS-CoV-2 infection in hospitalized patients with COVID-19: a randomized, parallel-group, open-label, multi-center pilot trial in the United States (PROTECT-COVID-19-US).

This clinical study protocol has been reviewed and approved by the Sponsor to ensure compliance with all regulations as described in the protocol.

Name	Signature	Date
Author, Clinical Project Manager: **Leisa Waynick**	Leisa Waynick	Jun 10, 2021
Chief Medical Officer: **Anurag Relan, MD**	Anurag Relan Anurag Relan (Jun 10, 2021 09:10 PDT)	Jun 10, 2021
Statistician: **Michael Bulitta**	Michael Bulitta Michael Bulitta (Jun 11, 2021 10:12 GMT+2)	Jun 11, 2021

Investigator at study site:

I have read and understood this study protocol and agree to conduct the study as set out in this study protocol, the Sponsor's standard operating procedures and/or guidelines, the United States Food and Drug Administration (FDA) regulations, the current version of the World Medical Association Declaration of Helsinki, the International Conference on Harmonization (ICH) GCP guidelines and other local regulations, as applicable.

Site: (Name and address of site)

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Site Investigator:

_____
Printed Name

_____ _____
Signature Date

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STUDY SYNOPSIS

Sponsor / Sponsor-Investigator	Pharming Technologies B.V.
Study Title:	Recombinant human C1 esterase inhibitor **(**rhC1INH) (Ruconest®) in the prevention of severe SARS-CoV-2 infection in hospitalized patients with COVID-19: a randomized, parallel-group, open-label, multi-center pilot trial in the United States (PROTECT-COVID-19-US)
Short Title / Study ID:	PROTECT-COVID-19-US: rhC1INH in the prevention of SARS-CoV-2 infection related deterioration
Protocol Version and Date:	Amendment 3, Version 4.0, Date 25 May 2021
Trial registration:	ClinicalTrials.gov NCT04530136
Study category and Rationale:	Clinical trial of medicinal product, category C investigating an IMP in the prevention of severe SARS-CoV-2 infection in hospitalized patients with COVID-19
Clinical Phase:	Phase 2a (therapeutic exploratory) trial

Background and Rationale:	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread globally since December 2019 causing a pandemic of Coronavirus disease 19 (COVID-19) in almost all countries worldwide. The clinical spectrum of COVID-19 ranges from asymptomatic carriers to respiratory failure requiring respiratory support in the intensive care unit (ICU). Systemic hyperinflammation is a hallmark of more severe stages of COVID-19 leading to acute respiratory distress syndrome ("ARDS"), mechanical ventilation and ultimately death. In this stage, COVID-19 is associated with a decrease in suppressor and regulatory T cell counts and an extensive release of proinflammatory cytokines and biomarkers called a cytokine storm, which is thought to be the major driver of severe pneumonia caused by SARS-CoV-2. No proven or approved effective treatment for COVID-19 infection currently exists. The mechanism responsible for virus-induced hyper-activation of the host immune system remains poorly understood but likely involves several immune cells and inflammatory plasmatic cascades such as the complement and the kinin-kallikrein (KK) system. The complement system (CS) is an integral part of the innate immune system and consists of a number of distinct plasma proteins that act as a first line of defense inducing an inflammatory response after opsonization of pathogens and dying cells. Previous evidence indicated that an over-activated complement systems, driven by the lectin pathway of complement in particular, seems to contribute to acute lung injury ("ALI") in response to infection with CoV such as SARS-CoV-2 leading to the clinical picture of severe COVID-19 pneumonia and consequently to ARDS. The KK system is a plasmatic cascade that after activation (shear stress of vessels, e.g. during vascular inflammation) and subsequent cleavage of kininogen by kallikrein releases bradykinin. Bradykinin binds to B2-receptors on endothelial cells leading to capillary leakage and angioedema. After enzymatic degradation bradykinin products may also bind to B1-receptors on endothelial cells that are upregulated under proinflammatory conditions and have strong vasopermeable capacity. Although direct evidence is lacking, several facts argue for an involvement of bradykinin in pulmonary angioedema observed in COVID-19, in particular a reduced activity of ACE2 caused by SARS-CoV-2 leading to a relative abundance of bradykinin degradation production with subsequent B1 activation and local pulmonary edema. C1 esterase inhibitor (C1INH) is a member of the serpin superfamily of serine-protease inhibitors and is a strong inhibitor of the CS and KK system among others. RhC1INH is a recombinant human C1INH, that shares an identical protein structure with plasma-derived C1INH. Although data on C1INH treatment in the context of SARS-CoV-2 infection are lacking, results from previous studies suggest that C1INH treatment may reduce the collateral damage caused by hyperinflammation in human sepsis. The rationale of the current trial is based upon the following assumptions: In the context of COVID-19, rhC1INH treatment may: 1) dampen

	uncontrolled complement activation and collateral lung damage and 2) reduce capillary leakage and subsequent pulmonary edema by direct inhibition of KK system. **Hypothesis:** Administration of rhC1INH for 4 days in addition to standard of care (SOC) in patients hospitalized with non-critical SARS-CoV-2 pneumonia (WHO Ordinal Scale Score 3 or 4) will be associated with a reduced clinical severity on day 7 after inclusion and a lower risk of disease progression to ALI and ARDS.
Objective(s):	The primary objective of the study is to determine if adding 4 days of treatment with rhC1INH to SOC treatment in adult participants admitted with non-critical COVID-19 will affect disease severity within 7 days after inclusion as assessed by the WHO Ordinal Scale for Clinical Improvement ("WHO Ordinal Scale"). Secondary objectives will determine if rhC1INH will - Reduce the time to clinical improvement (time from randomization to an improvement of two points on the WHO ordinal scale or live discharge from hospital, whichever came first) within 14 days after enrollment. - Increase the proportion of participants alive and not having required invasive or non-invasive ventilation at 14 days after enrollment. - Reduce the proportion of subjects with ARDS (defined by Berlin severity criteria) within 14 days after enrollment.
Outcome(s):	The primary endpoint will be the disease severity on the 7-point WHO Ordinal scale on day 7. The WHO Ordinal scale measures illness severity over time. Secondary endpoints include: - Time to clinical improvement (time from randomization to an improvement of two points on the seven-category WHO Ordinal scale or live discharge from hospital, whichever came first) within 14 days after enrollment. - Proportion of participants alive and not having required invasive or non-invasive ventilation at 14 days after enrollment. - Proportion of subjects with ARDS (defined by Berlin severity criteria) within 14 days after enrollment.
Study design:	Randomized, open-label, parallel-group, controlled, multi-center clinical trial

Inclusion / Exclusion criteria:	Patients admitted for the management of confirmed COVID-19 will be approached. Inclusion criteria: • Informed Consent as documented by signature, • Male or Female of age 18-85 years, • Admitted to the hospital because of confirmed (by a positive SARS-CoV-2 PCR result) COVID-19 infection, • Expected to remain an inpatient over the next four (4) calendar days from time of enrollment, • Evidence of pulmonary involvement on CT scan or X-Ray (optional/if available) of the chest, • Symptom onset within the previous 10 days or shortness of breath within the previous 5 days and • At least one additional risk factor for progression to mechanical ventilation: 1) arterial hypertension, 2) >50 years, 3) obesity (BMI>30.0 kg/m2), 4) history of cardiovascular disease, 5) chronic pulmonary disease, 6) chronic renal disease, 7) C-reactive protein of >35mg/L, 8) oxygen saturation at rest in ambient air of <94%. Cardiovascular disease includes a history of coronary artery disease, cerebrovascular disease, peripheral artery disease, rheumatic heart disease, congenital heart disease and of recent (< 3 months) deep vein thrombosis or pulmonary embolism. Chronic pulmonary disease includes a history of chronic obstructive pulmonary disease, asthma, occupational lung disease, interstitial lung disease or of pulmonary hypertension. Chronic renal disease is defined as a history of an estimated glomerular filtration rate (according to the Chronic Kidney Disease Epidemiology Collaboration equation) < 60ml/min/1.73 m2 for at least three months. *Also refer to the WHO definition for additional Cardiovascular diseases **Exclusion criteria:** • Contraindications to the class of drugs under study (C1 esterase inhibitor), *e.g*. known hypersensitivity or allergy to class of drugs or the investigational product, • Treatment with tocilizumab or another Il-6R or Il-6 inhibitor (within 24 hours) prior to enrollment, • History or suspicion of allergy to rabbits, • Women who are of childbearing potential and not using at least one method of contraception (oral contraceptives, barrier methods, approved contraceptive implant or abstinence) before randomization, after discharge and for the entire follow up study period, • Women who are pregnant or breast feeding or have a positive serum β-human chorionic gonadotropin (hCG) pregnancy test at screening, • Active or planned treatment with any other complement inhibitor,

	• Chronic Liver Disease (any Child-Pugh score B or C), • Incapacity or inability to provide informed consent, • Currently admitted to an ICU or expected admission within the next 24 hours, • Currently receiving invasive or non-invasive ventilation (with the exception of high-flow oxygen therapy), • In the opinion of the treating time, death is deemed to be imminent and inevitable within the next 24 hours, • Participation in another study with investigational drug within the 30 days preceding and during the present study with the following exemptions: 1) participation in COVID-19 drug trials started at least 48 hours before admission (e.g. postexposure prophylaxis with hydroxychloroquine) and 2) participation in COVID-19 drug trials during ICU admission, • Any uncontrolled or significant concurrent illness that would put the patient at a greater risk or limit compliance with the study requirements at the discretion of the investigator. • Previous enrollment into the current study, and • Enrollment of the Investigator, his/her family members, employees and other dependent persons

<table>
<tr><td>Measurements and procedures:</td><td>After providing informed consent a respiratory tract sample will be obtained. Only subjects fulfilling all eligibility criteria will be randomized in a 2:1 ratio in an open-label controlled design to treatment with rhC1INH in addition to SOC or SOC only starting on day 0. The first rhC1INH treatment will be administered on the same day and continued for a total of 4 days.
Vital signs, disease severity, clinical improvement, admission to ICU, receipt of additional anti-inflammatory therapies such as tocilizumab and requirement for non-invasive or invasive ventilation will be documented. Disease severity will be evaluated by the WHO Ordinal Scale:
<table>
<tr><td>Patient State</td><td>Description</td><td>Score</td></tr>
<tr><td rowspan="2">Outpatient</td><td>No limitation in activities</td><td>1</td></tr>
<tr><td>Limitation in activities</td><td>2</td></tr>
<tr><td rowspan="2">Hospitalized Mild disease</td><td>No oxygen therapy</td><td>3</td></tr>
<tr><td>Oxygen by mask or nasal prongs</td><td>4</td></tr>
<tr><td rowspan="2">Hospitalized Severe disease</td><td>Non-invasive ventilation or high-flow oxygen</td><td>5</td></tr>
<tr><td>Intubation, mechanical ventilation +/- additional organ support</td><td>6</td></tr>
<tr><td>Death</td><td>Death</td><td>7</td></tr>
</table>
Virological clearance will be assessed at 14 days after enrollment or discharge. Routine laboratory parameters and changes in certain biomarker levels will be assessed daily until discharge.
Follow-up will include assessment of adverse events and outcomes for 3 months post-treatment. This will be assessed daily until discharge and weekly via telemedicine interview or telephone call post-discharge.</td></tr>
<tr><td>Study Product / Intervention:</td><td>RhC1INH will be administered (150 U/ml) at a 50 U/kg dose (max dose of 4200 U) as a slow intravenous injection in approximately 5 minutes every 12 hours for 4 days. A total of 8 doses will be administered. Both trial arms will receive SOC treatment according to local guidelines.</td></tr>
<tr><td>Control Intervention (if applicable):</td><td>Standard of care treatment including supplemental oxygen, antibiotics, off-label drugs for the treatment of COVID-19, and treatment for comorbid conditions and underlying disease.</td></tr>
</table>

Number of Participants with Rationale:	Approximately 120 subjects (80 in the active treatment arm, 40 in the SOC group). The primary endpoint is a 7-point scale and the standard deviation σ can be expected as 1.5 points. A relevant effect δ is an advantage of at least 1 point. Then, the standardized difference is about δ/σ = 0.67. For a fixed sample size design with a two-sided significance level of α = 0.05 and a power of 1 - β = 0.80, a sample size of N = 2 x 38 is necessary. Because of a nonparametric test, we add 4 patients in each group, and because of drop-outs we add also 4 patients in each group to N = 2 x 46. For a 2:1-randomization, a nonparametric analysis by the stratified Wilcoxon rank-test, and an adaptive group sequential analysis, the overall sample size is estimated as 120 = 80 + 40. All patients are hospitalized, and it is very unlikely that the primary endpoint at Day 7 will not be measured. Nevertheless, a drop-out rate of about 10% is included. Two interim analyses after 32 and 80 patients are planned according to the Pocock adjusted levels αp = 0.0221. Based on the results of an interim analysis the sample size can be adjusted (Lehmacher, Wassmer, 1999). There are no prespecified futility margins, but the DSMB can stop the study in the case of insufficient interim results.
Study Duration:	Estimated 12 months
Principal Investigator(s):	Jonathan A. Bernstein, M.D.
Study Centre(s):	Multi-center
Statistical Considerations:	Detailed methodology for statistical analyses of the data collected in this trial will be documented in a Statistical Analysis Plan. Full Analysis Set/Intent-to-Treat Population: The FAS/ITT Population is defined as all patients who are randomly allocated to a study arm. Statistical analyses will be based on the treatment arm to which the patient was allocated. Safety Population: The Safety Population is defined as all patients who received at least one dose of rhC1INH. The primary endpoint WHO 7-point outcome scale at day 7 will be analyzed by means of the nonparametric Wilcoxon rank test stratified by its baseline values with two-sided α-level of 5 %. The secondary endpoint is time to improvement of at least 2 points. It will be tested only after a significant test of the primary endpoint (a priori ordered hypotheses), therefore, no alpha adjustment is necessary. Furthermore, 95% confidence intervals will be determined, and the results will be presented graphically by means of box-and-whisker plots. Time to event (e.g. death, virological clearance, defervescence) will be displayed by Kaplan-Meier plots and compared with a Wilcoxon rank test. Appropriate statistical tests will be used for the analysis of other outcomes of interest and will be detailed in the statistical analysis plan prior to unlocking of the study database.

GCP Statement:	This study will be conducted in compliance with the protocol, the Sponsor's standard operating procedures and/or guidelines, the United States Food and Drug Administration (FDA) regulations, the current version of the Declaration of Helsinki, the International Conference on Harmonization (ICH) GCP guidelines and other local regulations, as applicable.

ABBREVIATIONS

Provide a list of abbreviations used on the protocol - to be completed

AE	Adverse Event
ALI	Acute lung injury
C1-INH	C1 inhibitor, C1 esterase inhibitor
COVID-19	Coronavirus disease 2019
CRF	Case Report Form (Paper)
CS	Complement system
CTCAE	Common terminology criteria for adverse events
DSUR	Drug safety update report
EMA	European Medicines Agency
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HAE	Hereditary Angioedema
Ho	Null hypothesis
H1	Alternative hypothesis
HRI	Host-Related Impurities
IB	Investigator's Brochure
IC	Informed Consent
ICU	Intensive Care Unit
IME	Important Medical Event
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
ISO	International Organization for Standardization
ITT	Intention to treat
KK	Kinin-kallikrein
MD	Medical Device
PI	Principal Investigator
PV	Pharmacovigilance
rhC1INH	Recombinant human C1 esterase inhibitor
SAE	Serious Adverse Events
SARS-CoV-2	Severe acute respiratory syndrome-coronavirus-2
SDV	Source Data Verification
serpin	Serine Protease Inhibitor
SOC	Standard of Care
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
U/kg	U/kg units per kilogram body weight
US	United State of America
WHO	World Health Organization

1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor

Pharming Technologies B.V.
Darwinweg 24
2333 CR Leiden, The Netherlands
(Pharming Technologies B.V. is a subsidiary of Pharming Group N.V.)

1.2 Principal Investigator(s)

Jonathan A. Bernstein, M.D.

1.3 Data Safety Monitoring Committee

For rhC1INH serious adverse events see the current Investigator's Brochure (IB).
As the frequency of comorbidities in the patient population under study is significant and clinical complications are common in patients with COVID-19 infection, serious adverse events are possible in this setting. Hence, we will establish periodic interim safety review meetings (ISRM) by an independent committee. This Data Safety Monitoring Board (DSMB) will be responsible for safeguarding the interests of the study participants by monitoring adverse events and in particular serious adverse events. The DSMB may also consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the patients or the ethics of the study. An independent statistician will provide the DSMB with the pertinent safety data. The DSMB's responsibility, roles and procedures will be specified in the study-specific DSMB Charter. A first ISRM will take place after inclusion of 32 patients. The advice(s) of the DSMB will only be sent to Pharming (Sponsor). Should the Sponsor decide not to fully implement this advice, the Sponsor will send the advice to the Institutional Review Board (IRBs) and the Food Drug Administration (FDA), as appropriate, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

1.4 Any Other Relevant Committee, Person, Organization, Institution

Name(s) and address(es) of the clinical laboratory(ies) and other medical and/or technical department(s) and/or institutions involved in the trial:
Statistical Analysis and Data management: CRMB Biometrics GmbH
Data Safety Monitoring Board Committee: The University of Cincinnati
IMP Labeling/storage/dispensing: Cardinal Health & Cardinal Health Packing Solutions
Manufacturer of rhC1INH: Pharming Technologies, B.V. ("Pharming")

1.5 Study Sites

This will be a multicenter study with approximately 4 to 10 sites participating in the United States.

2. ETHICAL AND REGULATORY ASPECTS

Infection with SARS-CoV-2 is associated with moderate to severe disease requiring hospital admission in at least 20% of patients, and consequently about 20% of these admitted patients will deteriorate despite supportive treatment. Currently, evidence regarding specific therapies to treat SARS-CoV-2 or its associated inflammatory damage is still lacking. The current study will evaluate the safety and efficacy of rhC1INH treatment in COVID-19 patients at risk for clinical deterioration.

Although experimental data indicate a potential effect of rhC1INH, it remains unclear if recruited subjects will receive any immediate benefit from this research. However, the knowledge gained from this study may help develop effective treatment strategies for patients at high risk for clinical deterioration, which is important given the paucity of available effective treatment options.

All participants will receive standard supportive care established at the sites which may include off-label use of certain drugs that are deemed to be effective in COVID-19 (e.g., lopinavir/ritonavir). In addition, escalation of treatment with off-label use of certain drugs (e.g. tocilizumab) in deteriorating participants is allowed during the trial. Anaphylactic reactions are exceptionally rare and have only been observed in a single patient with a pre-existent allergy to rabbits. Although patients with rabbit allergy will be excluded from participating in the study, included subjects will be monitored for anaphylactic events during and for at least 12 hours after each administration of rhC1INH. A randomized, open-label study design was chosen due to the limited experience of rhC1INH in this setting. The majority of study assessments will be conducted while the participants are admitted. After discharge, the 3 month follow-up period will continue via weekly telemedicine interview or telephone call to assess adverse events and outcomes.

Participants' confidentiality will be maintained throughout the trial.

The decision of the FDA and the IRB concerning the conduct of the study will be made in writing to the Sponsor before commencement of this study. The clinical study can only begin once approval from all required authorities has been received. Any additional requirements imposed by the authorities shall be implemented.

2.1 Study Registration

The study will be registered at ClinicalTrials.gov.

2.2 Institutional Review Board (IRB)

The Principal Investigator and the responsible Investigator at each site ensure that approval from an appropriately constituted IRB will be sought for the clinical study prior to commencement of the study. No changes will be made to the protocol without prior IRB approval, except where necessary to eliminate apparent immediate hazards to study participants.

Reporting duties: Written progress reports will be sent to the IRB annually, or more frequently if requested by the IRB. All Suspected Unexpected Serious Adverse Reactions (SUSARS) will be reported within 7 (leading to death and life-threatening) or 15 (others) days to the IRB.

Premature Termination of the Study

Premature study end or interruption of the study is reported within 15 days. The regular end of the study is reported to the IRB within 90 days, the final study report shall be submitted within one year after study end. Amendments are reported according to Section 2.9.

Reasons for terminating the clinical study or participation of a study site may include, but are not limited to, the following:

- The incidence and severity of AEs in this or other studies indicates a potential hazard to patients.
- Patient enrollment is unsatisfactory.
- Data recording is inaccurate or incomplete.
- The Investigator of the study site shows serious and/or persistent non-compliance with the clinical study protocol (CSP), the clinical research agreement, and/or applicable regulatory guidelines while conducting the study.
- The (central) ethics committee decides to terminate or suspend approval for the clinical study or the Investigator.
- Investigator is accused of fraud (i.e., altered data, omitted data, or manufactured data).

In the event that the study is terminated prematurely, the Sponsor will provide specific guidance to study sites regarding end-of-study procedures.

2.3 Food Drug Administration (FDA)

The Sponsor will obtain approval from FDA before the start of the clinical trial. Written progress reports will be sent to the FDA annually, or more frequently if requested by FDA. All SUSARs will be reported within 7 (leading to death and life-threatening) or 15 (others) days to FDA. Unanticipated problems involving risks to humans will be reported immediately to FDA.

Premature study end or interruption of the study is reported within 15 days. The regular end of the study is reported to the IRB within 90 days, the final study report shall be submitted within one year after study end.

2.4 Ethical Conduct of the Study

The study will be carried out in accordance to the protocol and with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH and the FDA's requirements. The IRBs and regulatory authorities will receive annual safety and interim reports and be informed about study stop/end in agreement with local requirements.

2.5 Patient Information and Informed Consent

The Investigators will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each participant will be informed that the *participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of* consent will not affect his/her subsequent medical assistance and treatment.

The participant must be informed that his/her medical records may be examined by authorized individuals other than their treating physician.

All participants for the study will be provided a participant information sheet and a consent form describing the study and providing sufficient information for participant to make an informed decision about their participation in the study. Participants will have at least two hours and the opportunity to ask questions in order to decide on their participation. In the event that Informed Consent (IC) is obtained on the date that any study procedures are performed, the study record or subject´s clinical record will clearly show that IC was obtained prior to these procedures.

Due to the special situation in SARS-CoV-2 infected patients including physical isolation, consent will be obtained in two possible ways (in accordance with the *FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Public Health Emergency Guidance for Industry, Investigators, and Institutional Review Boards*):

Method 1: A photograph of the signed informed consent document can be transmitted to the trial staff

1. An unsigned consent form is provided to the patient by a person who has entered the room.
2. The Investigator/designee arranges a telephone call or video conference call with the patient (and, if desired and feasible, additional individuals requested by the patient (e.g., next of kin).

3. To ensure that patients are approached in a consistent fashion, a standard process should be used that will accomplish the following:

- Identification of who is on the call.
- Review of the informed consent document with the patient by the Investigator/designee and response to any questions the patient may have.
- Verbal confirmation by the patient that their questions have been answered that they would like to participate in the trial, and that they have signed and dated the informed consent document that is in their possession.

4. The patient (or an individual in the room) takes a photograph of the signed informed consent document and sends it to the Investigator/designee.

5. A trial team member enters the photograph into the trial records along with an attestation that states how that photograph was obtained and that it is a photograph of the informed consent document signed by the patient.

Method 2: A witness can attest to the signature, but a photograph of the signed informed consent document cannot be transmitted

1. An unsigned consent form is provided to the patient by a person who has entered the room.

2. The Investigator/designee arranges a three-way telephone call or video conference call with the patient, a witness who is not otherwise connected with the clinical investigation, and, if desired and feasible, additional individuals requested by the patient (e.g., next of kin). Alternatively, in lieu of using a witness, a recording of the conversation can be made.

3. To ensure that patients are approached in a consistent fashion, a standard process should be used that will accomplish the following:
   - Identification of who is on the call.
   - Review of the informed consent document with the patient by the Investigator/designee and response to any questions the patient may have.
   - Verbal confirmation by the patient that their questions have been answered that they would like to participate in the trial, and that they have signed and dated the informed consent document that is in their possession.

4. When using a witness, documentation in the trial records includes:
   - A signed and dated attestation by the witness who participated on the call that the patient confirmed their agreement to participate in the trial and signed the informed consent document, and
   - A signed and dated attestation by the Investigator/designee stating why the informed consent document signed by the patient was not retained (e.g., due to potential contamination of the document by infectious material).

The formal consent of a participant, using the approved consent form, must be obtained before the participant is submitted to any study procedure.

The participant should read and consider the statement before signing and dating the informed consent form and should be given a copy of the signed document. The consent form must also be signed and dated by the Investigator (or his designee) at the same time as the participant sign, and it will be retained as part of the study records.

2.6 Participant Privacy and Confidentiality

The Investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall

be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilizing subject identification code numbers to correspond to treatment data in the computer files.

For data verification purposes, authorized representatives of the Sponsor, or a competent authority (e.g. FDA) may require direct access to parts of the medical records relevant to the study, including participants' medical history.

2.7 Early Termination of the Study

The Sponsor may terminate the study prematurely according to certain circumstances, for example:

- ethical concerns,
- insufficient participant recruitment,
- when the safety of the participants is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- early evidence of benefit or harm of the experimental intervention

A DSMB will continuously monitor patients' safety. The DSMB will independently judge and recommend on the need to halt enrollment for further evaluations or to terminate the trial based on information regarding safety.

2.8 Protocol Amendments

The PI and Sub-Investigators are allowed to provide suggestions for a protocol amendment.

Substantial amendments are only implemented after approval of the IRB(s). Investigators will be notified immediately after approval and provided with updated study documents, and dossiers in trial registries updated accordingly.

Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the sponsor and the FDA and IRB. Such deviations shall be documented and reported to the sponsor and the FDA/IRB as soon as possible.

All non-substantial amendments are communicated to the FDA as soon as possible if applicable and to the IRB within the Annual Safety Report (ASR).

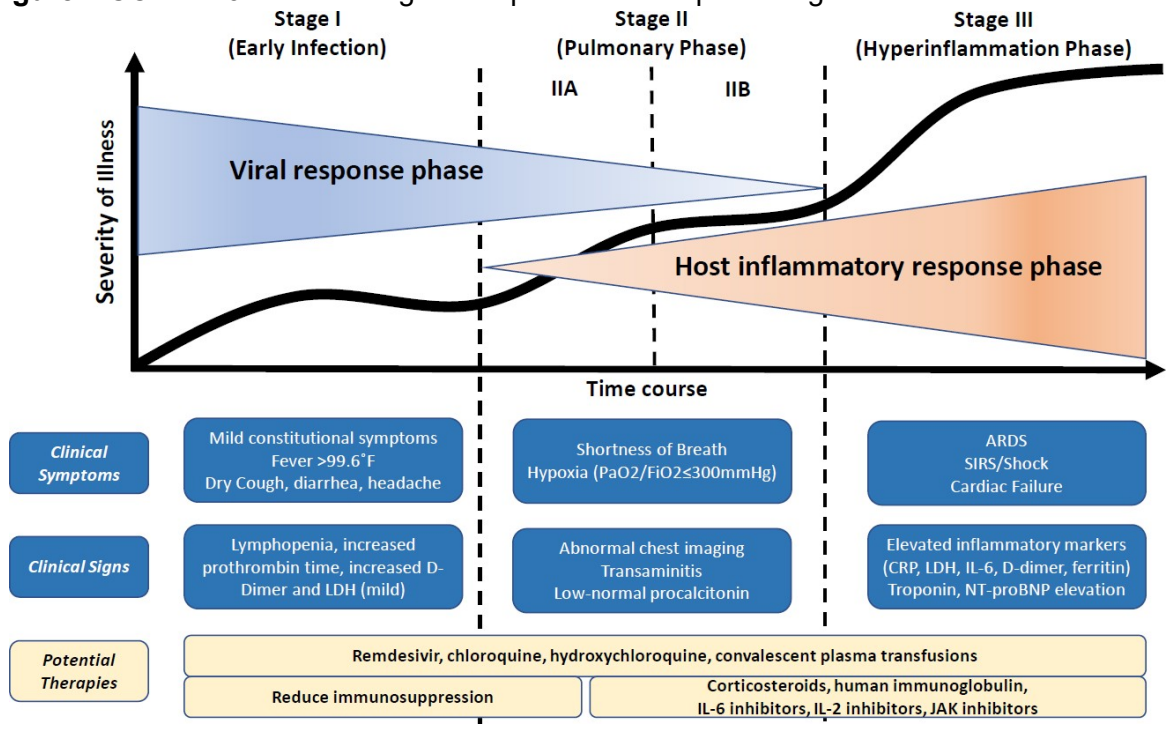
3. BACKGROUND AND RATIONALE

3.1 Background and Rationale

On 31 December 2019, a cluster of pneumonia cases of unknown etiology was reported in Wuhan, Hubei Province China. On 9 January 2020, China Center for Disease Control and Prevention (CDC) reported a novel coronavirus (2019-nCoV) as the causative agent of this outbreak, which is phylogenetically in the Severe Acute Respiratory Syndrome (SARS) coronaviruses (CoV) clade. It rapidly spread, resulting in an epidemic throughout China, followed by an increasing number of cases in other countries throughout the world. In February 2020, the World Health Organization (WHO) designated the disease COVID-19, which stands for coronavirus disease 2019. The virus that causes COVID-19 is designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of 14 April 2020 SARS-CoV-2 has spread globally with over 1,920,000 identified cases worldwide with over 119,000 deaths[2]. The WHO declared COVID-19 a global pandemic on March 2020.

SARS-CoV-2 is a single-stranded enveloped RNA virus, which targets cells through the viral structural spike protein that binds to the angiotensin-converting enzyme 2 (ACE2) receptor. The clinical spectrum of COVID-19 ranges from asymptomatic carriers to respiratory failure requiring respiratory support in the intensive care unit (ICU). In the largest report of COVID-19 from the Chinese CDC 81% of 72,313 cases were of mild nature (stage I)[3]. However, a subgroup of 5% presented with respiratory failure and multi-organ dysfunction leading to death in half of the cases (stage III).

Figure 1 COVID-19 disease stages and potential therapeutic regimen[4]



The exact reasons and factors promoting progression from stage I to stage III are not yet known. Stage I (mild infection) is characterized by infection and replication of SARS-CoV-2 in the respiratory system. In stage II (pulmonary involvement with (IIa) or without (IIb) hypoxia) patients develop viral pneumonia which is characterized not only by local viral replication but also by a localized inflammatory response. Clinically, patients present with fever, dry cough and dyspnea. Computerized tomography (CT) scans of the chest reveal uni- or bilateral ground-glass opacities, which are related to an increased fluid content in the alveoli of the lung. Affected individuals will usually require hospitalization during this stage. About 20% of stage II patients will progress into the most severe stage of illness (stage III), which is characterized

by a systemic hyperinflammatory syndrome leading to acute respiratory distress syndrome (ARDS). These patients usually require admission to the ICU for escalation of medial management including mechanical ventilation[5]. In this stage, COVID-19 infection is associated with a decrease in suppressor and regulatory T cell counts and an extensive release of proinflammatory cytokines and biomarkers such as interleukin (IL)-2, IL-6, ferritin, and D-dimer[6, 7], called a “cytokine storm”, which is thought to be the major driver of severe pneumonia caused by SARS-CoV-2.

No proven or approved effective treatment for COVID-19 infection currently exist. Several treatment candidates targeting the virus itself or systemic inflammation are under investigation in ongoing randomized trials. Given the high mortality rate in a subgroup of patients, there is clearly a need to develop management strategies to interrupt progression from stage I or II to stage III in COVID-19. The mechanism responsible for virus-induced hyper-activation of the host immune system remains poorly understood but likely involves several immune cells and inflammatory plasmatic cascades such as the complement and the kinin-kallikrein (KK) system.

The complement system (CS) is an integral part of the innate immune system and consists of a number of distinct plasma proteins that act as a first line of defense inducing an inflammatory response after opsonization of pathogens and dying cells[8, 9]. Inflammatory responses include the activation of macrophages, neutrophils, platelets and endothelial cells, interacting with other plasmatic cascades such as the coagulation cascade and direct cell injury, thereby increasing vascular permeability and tissue injury. The CS and particularly the lectin pathway of complement has been found to interact with and be involved in the clearance of several viruses[10-14]. While the CS does not seem to be critical for controlling CoV replication, unregulated complement activation - induced by viruses including influenza and CoV - plays a crucial role in the pathogenesis of acute lung injury (ALI). Indeed, an animal model suggests that the CS mediates SARS-CoV-induced lung disease and regulates the proinflammatory response. Complement deficient mice infected with SARS-CoV were affected less severely and showed a reduced lung involvement and lower local and systemic cytokine levels compared to control mice[15]. In line, inhibition of complement C5a signaling alleviated lung damage in animal infection models using MERS-CoV55[16] and an influenza H7N9[17]. Similar results were reported with inhibition of complement cascade C3a in an animal infection model using avian influenza[18]. Recently, Gao T *et al*. investigated the interaction of MERS-CoV, SARS-CoV and SARS-CoV-2 with the lectin pathway of complement in more detail[19]. They demonstrate an interaction of these highly pathogenic CoV with mannose-binding lectin associated serine protease-2 (MASP-2), the key activator of the lectin pathway of complement[20], leading to uncontrolled activation of the complement cascade. In line, MASP-2 knock-out mice and mice treated with MASP-2 inhibitors showed significantly milder symptoms in a virus protein mouse pneumonia model. Mannose-binding lectin, the pattern recognition molecule of the lectin pathway, that activates MASP-2 upon binding to pathogens, was found to bind to SARS-CoV spike glycoprotein[21]. Lastly, autopsy findings from a subgroup of patients with severe COVID-19 infection revealed excessive complement activation in the lung tissue associated with complement mediated microthrombotic disease[22]. Again, the lectin pathway of complement was implicated as major complement pathway in these patients. In summary, an over-activated complement system, driven by the lectin pathway in particular, seems to contribute to ALI in response to infection with CoV such as SARS-CoV-2 leading to the clinical picture of stage III COVID-19 infection and consequently to ARDS.

Besides the CS, the KK system may be involved in the pathogenesis of COVID-19, too. The KK system is a plasmatic cascade that after activation (shear stress of vessels, *e.g.* during vascular inflammation) and subsequent cleavage of kininogen by kallikrein releases bradykinin. Bradykinin binds to B2-receptors on endothelial cells leading to capillary leakage and angioedema. After enzymatic degradation bradykinin products may also bind to B1-receptors on endothelial cells that are upregulated under proinflammatory conditions and have strong vasopermeable capacity. Although direct evidence is lacking, several facts argue for

an involvement of bradykinin in pulmonary angioedema observed in COVID-19. ACE2 is not only a cell membrane bound protein that is utilized by SARS-CoV-2 to enter the cells but also possesses enzymatic activity inactivating bradykinin degradation products thereby preventing the activation of B1 receptor on endothelial cells. Interestingly, expression of ACE2 and its enzymatic activity is decreased in SARS-CoV and inflammatory conditions[23-25] and hence one may speculate that the interaction of SARS-CoV-2 with ACE2 may impair the function of ACE2 leading to a relative abundance of bradykinin degradation productions with subsequent B1 activation and local pulmonary edema[23].

Interestingly, there is a strong interaction between the CS and the KK system. For example, MASP-1, the potentiator of lectin pathway activation by MASP-2, was found to upregulate B2 receptors on endothelial cells[26]. Moreover, bradykinin release by MASP-1 mediated cleavage of kininogen was demonstrated[27]. Lastly, the enzyme that degrades bradykinin is identical to the inactivator of the complement anaphylatoxins C3a and C5a[28]. In summary, the KK system may be involved in COVID-19, particularly in stage II and III contributing to pulmonary edema and subsequently ARDS.

C1 esterase inhibitor (C1INH) is a member of the serpin superfamily of serine-protease inhibitors. It is an acute-phase protein that has manifold targets and biological functions, including inhibition of leucocytes and interactions with endothelial cells and microorganisms. C1INH is a strong inhibitor of the CS, factor XII and plasma kallikrein of the KK system. In particular, C1INH is the natural inhibitor of the lectin pathway of complement. MASP-1 and -2 seem to be the major target of C1INH with less effective inhibition of the classical pathway of the CS[29]. Decreased plasmatic antigenic levels of C1INH result in uncontrolled production of vasoactive peptides, which leads to the characteristic episodes of local soft tissue swelling observed in hereditary angioedema (HAE)[30]. C1INH deficiency seems to cause uncontrolled activation of MASP-1, which may aggravate HAE [31]. Currently, four C1INH preparations are available, three of them plasma-derived and one recombinant, *i.e.*, rhC1INH (rhC1INH, Ruconest®, Pharming, Leiden, The Netherlands). rhC1INH shares an identical protein structure with plasma-derived C1INH (pdC1INH) but has a different glycosylation pattern (containing abundant oligomannose residues), which is responsible for a shorter half-life than pdC1INH (3h vs. 30h)[32, 33]. Although comparable inhibition for most target proteases was demonstrated (including C1s, Factor XIa, XIIa and kallikrein)[33], rhC1INH seems to target the activation of the lectin pathway more effectively compared to plasma-derived preparations[34]. Despite the broad interference with several cascades and targets, major adverse events or unique toxicities have not been demonstrated in previous studies with the exception of a potential risk of allergic reactions in patients with rabbit dander allergy.

Although data on C1INH treatment in the context of SARS or influenza infections are lacking, results from pilot studies suggest that C1INH treatment may reduce the collateral damage caused by hyperinflammation in human sepsis[35-37]. Also, reduced occurrence of capillary leakage after allogeneic stem cell transplantation has been observed in a further study[38]. C1INH was also able to block MASP-2 mediated overactivation of the complement system induced by several CoVs[19].

In the context of COVID-19, rhC1INH treatment may: 1) dampen uncontrolled complement activation and collateral lung damage by inhibiting MASP-1 and MASP-2 in addition to classical pathway activation; and 2) reduce capillary leakage and subsequent pulmonary edema by direct inhibition of kallikrein activity and subsequent bradykinin release, inhibition of MASP-1 mediated upregulation of B2 receptors on endothelial cells and potentially reduced upregulation of B1 receptors on endothelial cells as a consequence of a reduced complement mediated inflammatory response.

Rationale of the Study

The rationale for rhC1INH is based on its mode of action, in particular its anti-inflammatory properties by inhibiting the CS and the KK system. Given the high percentage of sequence homology between SARS-CoV and SARS-CoV-2 similar complement and KK system dependent mechanisms may be involved in the susceptibility and severity of COVID-19[39]. Both plasmatic cascades most likely contribute to the inflammatory response after SARS-CoV-2 infection leading to ALI, ARDS and potentially death may be positively impacted by the therapy.

Given the above mentioned evidence, the lack of approved and effective treatment options for COVID-19, in particular for patients at risk of progression from stage II to stage III, the involvement of the CS and the KK system in ALI after infection with CoV and other respiratory viruses and the potent inhibition of both cascades by rhC1INH, a human trial to explore the effectiveness of rhC1INH in preventing the progression of COVID-19 infection in hospitalized non-critical ill patients is clearly desirable. The primary purpose of this study is to evaluate if adding rhC1INH to standard of care (SOC) in patients admitted for stage II COVID-19 infection may reduce the risk of disease progression, *i.e.* ALI requiring mechanical ventilation, or increase the chance of a faster clinical improvement compared to SOC alone. The treatment regimen is designed based on the timing and cause of the lung injury in COVID-19 infection and the role of the CS and KK system in the pathophysiology of COVID-19.

3.2 Investigational Product (treatment, device) and Indication

RhC1INH is purified from the milk of rabbits expressing the gene coding for human C1-INH.

RhC1INH is supplied as a sterile, preservative-free, white/off-white lyophilized powder for reconstitution for injection. Each vial contains 2100 units of rhC1INH. After reconstitution with 14 mL of sterile water for injection, each vial contains 150 U of rhC1INH per 1 mL in a 20 mM sodium citrate buffer with a pH of 6.8; vials are for single use only. The drug's shelf-life is 48 months at $\leq$25°C before reconstitution.

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

Recombinant Human C1 Esterase Inhibitor (Ruconest®) is licensed by the Food and Drug Administration (FDA) for the treatment of acute angioedema attacks in adults and adolescents with hereditary angioedema (HAE).

3.3 Preclinical Evidence

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 plasma-derived C1-INH, tested *in vitro*. In addition, although C1-INH can inhibit plasmin, tPA and thrombin, inhibition is weak. The expected increases of C1-INH plasma concentrations in the proposed clinical study do not have a significant effect on the activity of the proteases and are unlikely to affect the balance between clotting and fibrinolysis. Please refer to the IB for further information.

In the nonclinical program no concerns were found with respect to tolerability, cardio-respiratory safety, embryofetal toxicity, local tolerance, or immunogenicity of rhC1INH.
Acute and repeat dose toxicology and safety pharmacology as well as local tolerance studies in rats, dogs and rabbits support the safety-in-use of the rhC1INH preparation.

The acute studies showed no overt toxicological effects in rats and dogs with a single dose up to 1250 U/kg. A No-Observed-Adverse-Effect-Level (NOAEL) was established at 625 U/kg when dosed by continuous IV infusions for 14 days in rats and by daily IV infusions (5 mL/min) for 5 days in dogs. In the 14-day pivotal repeat dose toxicity study in monkeys, the NOAEL was established at twice daily IV infusions of 1000 U/kg.

The total dose of 2000 U/kg/day in this monkey study given for 14 consecutive days, exceeds the intended total clinical doses of 50 U/kg, 100 U/kg a day by 40 to 20-fold without adverse effects.

In a safety pharmacology study in dogs (dosed at 625 U/kg, 12.5- to 6.25-fold the intended total clinical dose of 50 U/kg / administration and 100 U/kg per day), no overt effects on vital functions such as the cardiovascular or respiratory system were observed. The established NOAEL was 625 U/kg.

RhC1INH is predominantly cleared from the circulation by liver receptors. It is more rapidly cleared from the circulation in rats, dogs and cynomolgus monkeys in comparison with plasma-derived C1-INH. This faster clearance likely results from the differential glycosylation, including a lower degree of sialylation in rhC1INH. Degradation is expected to be by proteolysis/hydrolysis within intracellular lysosomes. From studies in rats, dogs, and cynomolgus monkeys, it can be concluded that rhC1INH distribution is limited to the vascular compartment. For further information, see the IB.

RhC1INH has not been assessed in preclinical models of COVID-19 or related CoV infections. However, pdC1INH treatment was shown to reduce the activation of the CS and KK system in a large-animal model of septic shock. Importantly, release of proinflammatory cytokines such as TNF-α and IL-6 was attenuated [40]. In addition, administration of rhC1INH reduced lung tissue damage in an animal model of hemorrhage induced systemic inflammation. In particular, rhC1INH decreased tissue complement activation and deposition and circulating proinflammatory cytokines[41].

3.4 Clinical Evidence to Date

RhC1INH has not been assessed in patients with COVID-19 or related CoV infections in a clinical trial setting. RhC1INH has been extensively investigated in the treatment of acute attacks in patients with hereditary angioedema having demonstrated efficacy and safety (please, see IB for further details). In addition, a recent randomized, double-blind, placebo-controlled pilot study has demonstrated its efficacy and safety regarding attenuation of acute renal injury in patients undergoing elective coronary angiography[42].

With regards to infection and inflammation, pdC1INH treatment reduced the collateral damage caused by hyperinflammation in human sepsis in several small pilot studies[35-37]. In particular, it decreased complement and neutrophil activation[43]. Also, a reduced occurrence of capillary leakage after allogeneic stem cell transplantation has been observed in a further study in line with a decrease in the complement C5 activation product C5a[38, 44].

We have recently treated five patients admitted with stage II COVID-19 infection with compassionate use rhC1INH in addition to SOC in order to limit pulmonary inflammation and subsequent deterioration. RhC1INH was administered every twelve hours over 48 hours starting with a loading dose of 8400 U and continuing with 4200 U. RhC1INH was well tolerated with no treatment-emergent adverse events. Three patients had an immediate favorable response with rapid defervescence and decline in inflammatory markers and were discharged within a week. Two patients were administered additional treatment with tocilizumab and recovered afterwards, although one of these two patients rapidly progressed after 24 hours of treatment requiring transient mechanical ventilation.

3.5 Dose Rationale / Medical Device: Rationale for the Intended Purpose in Study (pre-market MD)

Pharmacological Characteristics
A phase 1, dose-escalating study in 12 asymptomatic patients with HAE demonstrated that rhC1INH administration provides a dose-dependent restoration of complement homeostasis. Patients with functional C1-INH levels <40% and C4 levels <300 µg/mL (values expectable in patients with HAE) received 2 IV doses of rhC1INH (range 6.25 to 100 U/kg), with a washout period of ≥5 weeks. A dose-dependent, biologic activity was demonstrated by increased plasmatic levels of C4, which is a substrate for activated C1s: 12 hours after rhC1INH administration (100 U/kg), the mean plasma levels of C4 were approximately double compared to baseline. Comparable results were shown after the 50 U/kg dose of rhC1INH, which was associated with a maximum C4 level of approximately 1.76-fold that at baseline, achieved after 10.7 hours.

A pharmacokinetic modelling indicated that a single IV injection of rhC1INH at a dose of 50 U/kg restores functional C1-INH levels to at least the lower limit of the normal range in almost all patients[45].

Following the slow IV administration (15 min) of 50 U/kg of rhC1INH to 6 asymptomatic patients with HAE, the mean maximum plasmatic level of functional C1-INH was 1.36 U/mL, mean volume of distribution was similar to plasma volume (approximately 3 L), mean elimination half-life was 2.4 hours and clearance was approximately 23 mL/min[46].
The pharmacokinetic data derived from the phase 1 studies demonstrated that the half-life of rhC1INH is shorter than plasma-derived C1-INH which is due to the different glycosylation of rhC1INH, leading to more rapid hepatic clearance compared to plasma-derived C1-INH.

In a phase 1 study in healthy volunteers, patients were administered rhC1INH on 5 occasions with intervals of at least 3 weeks. 14 patients received a total of 59 administrations of rhC1INH at a dosage of 100 U/kg. The mean elimination half-life was 2.7 hours and the baseline corrected Cmax was 2.6 U/mL. These values were comparable to those estimated in other studies. The course of functional C1-INH levels after infusion of 100 U/kg of rhC1INH was identical after 1st, 3rd and 5th administration.

RhC1INH is cleared via mannose/asialoglycoprotein receptors on macrophages and hepatic cells with carbohydrate recognition. RhC1INH is not expected to be excreted, but to be fully eliminated by degradation. As there are no clinical data with rhC1INH in patients with hepatic impairment, and as hepatic impairment may prolong the plasma half-life of rhC1INHrhC1-INH, patients with chronic liver disease will be excluded from study participation.

Safety
The clinical safety data analysis demonstrated that rhC1INH at doses of 50 and 100 U/kg are generally safe and well tolerated when administered for treatment of acute attacks in patients with HAE[47]. The adverse event profile found in the randomized, placebo-controlled studies was similar for patients treated in the rhC1INH and saline treatment groups.

Throughout the clinical development, no safety signal related to hematology, biochemistry, coagulation, urinalysis, vital signs, or ECG parameters was noted. Evidence regarding a thrombogenic risk of rhC1INH has not been recorded during clinical development and clinical studies. RhC1INH had no effect on activation of coagulation and fibrinolysis[48].

Because rhC1INH is derived from the milk of transgenic rabbits and contains a small percentage (0.002%) of Host-Related Impurities (HRI), immunogenicity was extensively tested

throughout the clinical development programs, leading to the evidence that rhC1INH has low potential to induce anti-C1-INH antibodies or anti-HRI response. The use of the product in patients allergic or suspected to be allergic to rabbits should be avoided because of the risk of an allergic reaction.

Sporadic, transient immune responses to rhC1INH and HRI were observed, but with no associated clinical findings. No patient developed neutralizing antibodies to C1-INH. No impact of immunogenicity on clinical efficacy or safety was observed.

There was no plausible temporal association between treatment-emergent adverse events or new acute attacks and the presence of any confirmed anti-C1-INH or anti-HRI antibodies in patients with HAE.

No patients tested positive for neutralizing antibodies to endogenous C1-INH or rhC1INH and no induced anti-rabbit IgE antibodies were reported. Healthy patients with clinically manifest rabbit allergy were administered rhC1INH by skin prick and – in case of lack of allergic response – an intradermal and subcutaneous injection. Two out of twenty patients revealed a positive response, suggesting that even in patients with a pre-existing allergy to rabbits the risk of a hypersensitivity reaction upon administration of rhC1INH is limited.

Lastly, safety was favorable with no related or treatment-emergent adverse events in a recent double-blind, placebo-controlled trial in elderly patients undergoing elective coronary angiography[42].

Description and Justification of Route of Administration and Dosage
Because C1-INH is a plasma glycoprotein, an IV route of administration has been chosen for the existing indication (acute attacks in patients with HAE). The same route of administration will be used in this study.

The approved dose for treatment of acute attacks in patients with HAE is 50 U/kg (up to 4200 U), with an option to repeat the dose once based on clinical symptoms. Studies on prophylactic usage at a dose of 50 U/kg (up to 4200 U) twice weekly, up to 4 weeks, to treat patients with HAE with frequent acute attacks appeared efficacious and well tolerated. Maximum doses (4200 U or 8400 U) have been administered to patients weighing 84 kg or more.

In the current study, rhC1INH (150 U/ml) at a 50 U/kg dose (max dose of 4200 U) will be administered as a slow IV injection over a period in approximately 5 minutes every 12-hours for 4 days. The dosage regimen was chosen based on the following rationale

- Although C1INH antigenic concentrations are often elevated in patients with infections (C1INH is an acute phase reactant), the functional activity may be compromised as a consequence of modified/cleaved and consequently inactive C1INH[49].
- The half-life of rhC1INH is 2.5 hours, hence in order to achieve a sustained inhibition of the CS and KK system, repeated administration has been chosen. Despite the short half-life sustained inhibition of the target proteases may be guaranteed related to the irreversible inhibition of target proteases followed by clearance from the bloodstream. In order to balance effectivity with practicability and potential toxicity we decided to limit the total daily dose and administer rhC1INH every 12 hours.
- Repeated administration of rhC1INH over four days has been chosen, as hyperinflammation may develop several days after the admission, in particularly during the time of seroconversion (development of antibodies against SARS-CoV-2)[50] Anti-inflammatory strategies, such as the current proposed treatment regimen with rhC1INH may have a particular role at the time of induction of hyperinflammation. Hence, treatment for at least 4 days is warranted.

Participants will either receive rhC1INH with SOC or only SOC.

3.6 Explanation for Choice of Comparator

Currently, no approved or effective treatment options for COVID-19 infections exist. Participants will either receive rhC1INH plus SOC or only SOC. SOC may include treatment for underlying comorbidities and management of COVID-19 symptoms. Importantly, local treatment protocols including drugs with perceived benefits in COVID-19 infections such as lopinavir/ritonavir are allowed as long as they are declared as SOC. In order to facilitate patient recruitment in a time of restricted human and financial resources, and because of an objective primary endpoint, a placebo group will be omitted.

Diphenhydramine, hydroxychloroquine, and azithromycin are associated with prolongation of the cardiac QT interval, and subsequent increased risk of fatal cardiac arrhythmia.

Cardiovascular monitoring will be conducted in severely-ill subjects receiving multiple drugs on a case-by-case adjudication of any prolongation of QT intervals to evaluate the benefit-risk of continued participation in the clinical study.

3.7 Risks / Benefits

Despite strong reasons for a benefit of rhC1INH in the setting of hyperinflammation, it is unclear if participants in the current study will receive any immediate benefit from treatment with rhC1INH. However, the knowledge gained from this study may help to better understand the pathophysiology of ALI and hyperinflammation in SARS-CoV-2 and related CoV infections. In addition, this trial may serve as pilot study for future rhC1INH studies in patients with severe infections characterized by hyperinflammation and subsequent tissue damage.

Previous experimental models have not suggested a detrimental effect of complement inhibition on viral replication, and C1-INH treatment has not been associated with an increased risk for secondary bacterial or viral infections in humans.

RhC1INH is approved for the treatment of acute angioedema attacks in adult and adolescent patients with HAE in the EEA countries and the United States and for adults in South Korea and Israel. Up to October 2019, 268 unique patients have been exposed to rhC1INH in the rhC1INH clinical development program and 3717 patients worldwide have been commercially prescribed rhC1INH without any change to the risk-benefit profile.

To date, the only known relevant clinical risk associated with rhC1INH is the possibility of an allergic reaction to Host-Related Impurities (rabbit). Patients with medical history of allergy to rabbits or rabbit-derived products including rhC1INH or suspicion of such an allergy, are excluded from participation.

To minimize the risk for allergic reactions to rhC1INH, treated patients will be monitored for clinical symptoms of hypersensitivity during and after dosing. All treatments will be administered in a hospital and all patients will stay under close observation until discharge from the hospital.

RhC1INH was demonstrated to be a safe option for reducing the risk of contrast-induced renal damage in high-risk patients[42]. In addition, rhC1INH was safely administered to five patients suffering from stage II COVID-19 infection.

Expected Adverse Events During the Study

As per last the Investigator's Brochure, expected adverse reactions are described as follows: The clinical development program supporting safety of rhC1INH is based on a total number of 268 unique patients (up to Oct 2019). Those 268 patients received a combined total of almost 1600 administrations of rhC1INH. The treatment emergent adverse events (TEAEs) that occurred with onset within 7 days of treatment with rhC1INH that were most frequently reported (observed in ≥3% of patients treated with rhC1INH) were headache (11%), nausea, and

diarrhea (3% each). There was no evidence of an increase in the percentages of patients with TEAEs or related TEAEs, or in the seriousness or severity of TEAEs, with increases in rhC1INH dose, treatment of a larger number of attacks, and/or treatment of a single attack with an additional dose.

As described in the US prescribing information for rhC1INH, AEs of headache, sneezing, angioedema, erythema marginatum, skin burning sensation, back pain, C-reactive protein increased, fibrin D-dimer increased, vertigo, lipoma, nausea, and diarrhea occurred in ≥2% of rhC1INH -treated patients. Note that AEs are listed in the US prescribing information regardless a causal relationship with rhC1INH. The frequency of adverse reactions listed above is defined using the following convention:

- Very common (≥1/10),
- Common (≥1/100 to <1/10),
- Uncommon (≥1/1,000 to <1/100),
- Rare (≥1/10,000 to <1/1,000),
- Very rare (<1/10,000),

Specific antidotes against rhC1INH do not exist. In the unlikely event of an allergic reaction, standard treatment regimens indicated by the clinical situation (*e.g*. epinephrine, antihistamines, corticosteroids, etc.) will be administered.

The level of risk may evolve over time, during the study: consequently, a Data Safety Monitoring Board (DSMB) will monitor the safety of all patients, as well as the continuing validity and scientific merit of the study.

3.8 Justification of Choice of Study Population

Approximately 20% of patients with COVID-19 infection require hospitalization for supportive treatment. The majority of these patients will suffer from viral pneumonia visible on chest CT scans or Chest X-Ray. Approximately 15-20% of these patients will progress to severe ALI requiring mechanical ventilation and ICU support. Patients with confirmed COVID-19 disease (based on a positive SARS-CoV-2 PCR result) and admitted to a non-ICU ward will be eligible for the study. This population was chosen, as anti-inflammatory treatments such as rhC1INH may interfere most effectively during stage II disease, *i.e*. during the early inflammatory response phase. In addition, interventions that prevent patients from deteriorating and requiring mechanical ventilation are highly desired and represent an area of unmet clinical need in a pandemic situation with limited ICU and ventilation support capacity. This population offers the opportunity to impact not only on an individual but also on a population level.

4. STUDY OBJECTIVES

4.1 Overall Objective

The purpose of this study is to determine whether rhC1INH administered early during hospital admission for COVID-19 provides additional benefit compared to SOC alone with regards to the clinical course of patients by limiting the local and systemic inflammatory response, and to describe its safety profile in this population.

4.2 Primary Objective

The primary objective of the study is to determine if adding 4 days of treatment with rhC1INH to SOC treatment in adult participants admitted with non-critical COVID-19 infection will affect disease severity including progression to severe disease requiring mechanical ventilation within 7 days after enrollment as assessed by the WHO Ordinal Scale for Clinical Improvement.

4.3 Secondary Objectives

To evaluate the effect of rhC1INH treatment in addition to SOC treatment compared to only SOC treatment on disease progression as measured by

- Time to clinical improvement (time from randomization to an improvement of two points on the seven-category WHO ordinal scale or live discharge from hospital, whichever came first) within 14 days after enrollment.
- Proportion of participants alive and not having required invasive or non-invasive ventilation at 14 days after enrollment.
- Proportion of subjects with ARDS (defined by Berlin severity criteria) within 14 days after enrollment

4.4 Safety Objectives

The study aims to assess the safety of rhC1INH treatment in patients admitted with COVID-19 infection and receiving SOC treatment including overall incidence of adverse and serious adverse events and their relationship to the study treatment during a 3 month follow-up period.

5. STUDY OUTCOMES

5.1 Primary Outcome

The primary endpoint will be the disease severity on the 7-point WHO Ordinal Scale on Day 7. This endpoint has been suggested by WHO for clinical trials in patients with COVID-19. The ordinal scale measures illness severity over time.

Figure 2: WHO ordinal scale for clinical improvement.

Patient State	Description	Score
Outpatient	No limitation in activities	1
	Limitation in activities	2
Hospitalized Mild disease	No oxygen therapy	3
	Oxygen by mask or nasal prongs	4
Hospitalized Severe disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation, mechanical ventilation +/- additional organ support	6
Death	Death	7

5.2 Secondary Outcomes

The secondary endpoints will evaluate the effect of rhC1INH treatment in addition to SOC treatment relative to only SOC treatment on disease progression as measured by

- Time to clinical improvement (time from randomization to an improvement of two points on the seven-category WHO Ordinal Scale or live discharge from hospital, whichever came first) within 14 days after enrollment.
- Proportion of participants alive and not having required invasive or non-invasive ventilation at 14 days after enrollment.
- Number of days hospitalized (per patient)
- Number of days alive (per patient)

- Proportion of subjects with ARDS (defined by Berlin severity criteria) within 14 days after enrollment

5.3 Other Outcomes of Interest

- Changes on the WHO Ordinal Scale from baseline over 14 days
- Length of hospital stay until day 28 in survivors.
- Proportion of participants progressing to mechanical ventilation on day 7 and day 14
- Proportion of participants requiring ICU treatment on day 7 and 14
- Length of ICU stay until day 28
- Ventilator-free days until day 28
- All-cause mortality (time from randomization to death within 4 weeks)
- Changes in biomarker levels until day 14: CRP, LDH, D-dimer, ferritin, IL-6, lymphocyte count
- Time to virological clearance of SARS-CoV-2 by PCR from upper or lower respiratory tract samples (time from enrollment to first of 2 negative assays at least 12 hours apart)
- Proportion of patients receiving additional anti-inflammatory treatment such as tocilizumab or immunoglobulins within 14 days
- Time to defervescence (temperature <38.0°C sustained for at least 48 hours)
- Time to clinical improvement (defervescence, normalization of oxygen saturation (>93%) and respiratory rate) until day 28
- Duration of supplemental oxygen until day 28
- In a subgroup of patients, the pharmacokinetics and pharmacodynamics of rhC1INH in COVID-19 patients will be characterized by measuring the concentration of rhC1INH and the activity of C1-INH and proteins (such as C3, C4).

5.4 Safety Outcomes

The study will evaluate the safety of rhC1INH in the setting of COVID-19 infections if added to SOC compared to SOC treatment only by measuring the incidence of adverse events up to 3 months post-treatment.

6. STUDY DESIGN

6.1 General Study Design and Justification of Design

This is an exploratory, randomized, parallel-group, open-label, multi-center, phase 2 clinical study in patients with confirmed COVID-19 infection and admitted to the hospital for treatment of SARS-CoV-2 infection to estimate the effect size of rhC1INH treatment compared to SOC on the proportion of patients surviving without requiring mechanical ventilation.

The study will recruit approximately 120 patients (80 patients in the intervention and 40 in the control arm) from approximately five to ten sites in the United States. Sites will be selected on the basis of estimated numbers of cases, the availability of a committed principal site Investigator and research team, and the capacity to collect samples as per protocol.

Participants will be randomly assigned in a 2:1 ratio to rhC1INH treatment in addition to standard of care (SOC) or SOC stratified by the site. Screening, informed consent and randomization will happen as early as possible after admission to the hospital, usually within 48 hours after admission. Participants will receive intravenous rhC1INH in addition to standard of care (SOC) or SOC during an admission period starting on the day after informed consent (=day 0). Both groups will continue to receive SOC treatment for COVID-19 infection. Blood samples will be collected before and during the 4-day period of treatment. Follow-up will include the period until discharge and a weekly telemedicine interview or telephone call for 3 months post treatment to assess potential adverse events and outcomes.

Duration of participant's participation will be approximately 14 weeks (+/- 5 days). Screening phase duration is up to 48 hours; intervention period is 14 days (including a treatment phase of 4 days) and follow-up period of 3 months starting post-treatment on Day 0. After discharge, follow-up information is obtained on a weekly basis until 3 months post-treatment. When no response is received within 5 weeks following discharge the participant may be considered as lost to follow-up.

6.2 Methods of Minimizing Bias

Unmeasured confounding and bias will be minimized by randomization and objective endpoints.

6.2.1 Randomization

Participants will be randomized to two parallel groups in a 2:1 ratio to receive either rhC1INH (intervention) in addition to standard of care (SOC) or SOC treatment (control). Randomization will be stratified by the study site before inclusion using randomly permuted block sizes of four.

6.2.2 Blinding Procedures

Participants, treating physicians and nurses and Investigators will not be blinded.

6.2.3 Other Methods of Minimizing Bias

Not applicable.

6.3 Unblinding Procedures (Code break)

Not applicable.

7. STUDY POPULATION

Patients admitted for the management of confirmed COVID-19 infection will be approached. If enrollment goals are not met expansion to other centers in the United States will be explored.

7.1 Eligibility Criteria

Participants fulfilling all of the following inclusion criteria are eligible for the study:

- Informed Consent as documented by signature,
- Male or Female age 18-85 years,
- Admitted to the hospital because of confirmed COVID-19 infection (by a positive SARS-CoV-2 PCR result),
- Expected to remain an inpatient over the next four (4) calendar days from time of enrollment,
- Evidence of pulmonary involvement on CT scan or chest X-Ray of the chest (e.g. ground glass opacities),
- Symptom onset within the previous 10 days OR shortness of breath within the previous 5 days. Symptoms include fever or one respiratory symptom (patients presenting later may have already progressed to an inflammatory state that is potentially not amenable to C1INH treatment). Respiratory symptoms include cough, sore throat, hemoptysis, shortness of breath, runny nose, or chest pain and
- at least one additional risk factor for progression to mechanical ventilation: 1) arterial hypertension, 2) >50 years, 3) obesity (BMI>30.0 kg/m2), 4) history of cardiovascular disease, 5) chronic pulmonary disease, 6) chronic renal disease, 7) C-reactive protein of >35mg/L, 8) oxygen saturation at rest in ambient air of <94%. Cardiovascular disease includes a history of coronary artery disease, cerebrovascular disease, peripheral artery disease, rheumatic heart disease, congenital heart disease and of

recent (< 3 months) deep vein thrombosis or pulmonary embolism. Chronic pulmonary disease includes a history of chronic obstructive pulmonary disease, asthma, occupational lung disease, interstitial lung disease or of pulmonary hypertension. Chronic renal disease is defined as a history of an estimated glomerular filtration rate (according to the Chronic Kidney Disease Epidemiology Collaboration equation) < 60ml/min/1.73 m2 for at least three months.

*Also refer to the WHO definition for additional Cardiovascular diseases

The presence of any one of the following exclusion criteria will lead to exclusion of the participant:

- Contraindications to the class of drugs under study (C1 esterase inhibitor), *e.g*. known hypersensitivity or allergy to class of drugs or the investigational product,
- Treatment with tocilizumab or another Il-6R or Il-6 inhibitor (within 24 hours) prior to enrollment,
- History or suspicion of allergy to rabbits,
- Women who are of childbearing potential and not using at least one method of contraception (oral contraceptives, barrier methods, approved contraceptive implant or abstinence) before randomization, after discharge and for the entire follow up study period,
- Women who are pregnant or breast feeding or have a positive serum β-human chorionic gonadotropin (hCG) pregnancy test at screening,
- Active or planned treatment with any other complement inhibitor,
- Chronic Liver Disease (any Child-Pugh score B or C),
- Incapacity or inability to provide informed consent,
- Currently admitted to an ICU or expected admission within the next 24 hours,
- Currently receiving invasive or non-invasive ventilation, (with the exception of high-flow oxygen therapy).
- In the opinion of the treating time, death is deemed to be imminent and inevitable within the next 24 hours,
- Participation in another study with investigational drug within the 30 days preceding and during the present study with the following exemptions:
  1) participation in COVID-19 drug trials started at least 48 hours before admission (e.g. postexposure prophylaxis with hydroxychloroquine) and
  2) participation in COVID-19 drug trials during ICU admission,
- Any uncontrolled or significant concurrent illness that would put the patient at a greater risk or limit compliance with the study requirements at the discretion of the investigator,
- Previous enrollment into the current study, and
- Enrollment of the Investigator, his/her family members, employees and other dependent persons

7.2 Recruitment and Screening

Patients who are admitted to the hospital for treatment of confirmed COVID-19 infection will be screened after results from history (symptom onset) and routine investigations (including a CT scan or Chest X-Ray of the chest) if available. Patients that at least fulfil the age, CT or chest x-ray criteria and reason for admission will be approached by study staff to explain the study, verify inclusion/exclusion criteria and obtain informed consent prior to enrollment.

7.3 Assignment to Study Groups

Following study enrollment, an Investigator or study nurse will determine treatment allocation via the randomization list. Subsequently, rhC1INH will be prepared and supplied by the local pharmacy to the Investigator or study nurse for administration in the active treatment group.

7.4 Criteria for Withdrawal / Discontinuation of Participants

Subjects may voluntarily withdraw from study participation at any time without having to provide a reason. Subjects may be withdrawn because of the appearance of a new health condition requiring care or medications prohibited by the protocol, unacceptable adverse event, refusal to continue treatment, or at the Investigator's discretion if it is in the subject's best interest.

A subject who withdraws informed consent before randomization or who develops a violation of the selection criteria before randomization is defined as a screening failure. No follow-up of screening failures will be performed.

Participants who withdraw informed consent, who do not fulfil inclusion/exclusion criteria after obtaining informed consent or who are diagnosed with an alternative disease (e.g. influenza infection) AND have not received any study medication will be withdrawn from the study.

Participants who experience a type I allergic reaction after any dose of study medication will be discontinued from further study interventions. Withdrawn or discontinued participants will not be replaced. Follow-up for all patient groups will be explained in Section 8.5.

8. STUDY INTERVENTION

8.1 Identity of Investigational Products (treatment / medical device)

8.1.1 Experimental Intervention (treatment)

RhC1INH (Ruconest®) will be supplied by the production company Pharming Technologies, B.V., the Netherlands. RhC1INH is a recombinant analogue of human C1-INH for intravenous injection. The primary and secondary structures of the molecule and target protease selectivity are consistent with those of plasma-derived C1-INH. RhC1INH is purified from the milk of transgenic rabbits, and supplied as a sterile, preservative-free, white/off-white lyophilized powder for reconstitution for injection. RhC1INH contains less than 0.002% of rabbit-related impurities. One international unit (U) of rhC1INH activity is defined as the equivalent of C1-INH activity present in 1 mL of pooled normal plasma. Each vial of RhC1INH contains 2100 U of rhC1INH, 937 mg of sucrose, 83.3 mg of sodium citrate dihydrate and 1.0 mg of citric acid monohydrate. After reconstitution with 14 mL of sterile water for injection, each vial of rhC1INH contains 150 U of rhC1INH per 1 mL in a 20 mM sodium citrate buffer with a pH of 6.8. RrhC1INHRrhC1-INH does not contain preservatives and each vial is for single use only. After randomization, a pharmacist will open the respective sealed boxes, will reconstitute the respective number of rhC1INH vials and prepare the study medication. RhC1INH is for intravenous use only. The reconstituted solution is administered as a slow intravenous injection over approximately five minutes. Recommended doses of rhC1INH for the treatment of an acute angioedema attack are 50 U/kg if body weight <84kg and 4200 U if >84kg. A second dose may be administered at the same recommended dose level within a 24-hour period. In this study, we will not use the product according to the licensed indication. A market batch will be used for the study.

8.1.2 Control Intervention (standard/routine/comparator treatment / medical device)

Participants in the control arm will receive SOC treatment according to local guidelines.

8.1.3 Packaging, Labelling and Supply (re-supply)

The investigational product (rhC1INH) is supplied in single-use 24ml glass vials with a stopper (siliconized chlorobutyl rubber) and a flip-off seal (aluminium and colored plastic). Each carton contains one single-use vial. Sufficient amounts of rhC1INH will be supplied by the manufacturer Pharming Technologies B.V. prior to study commencement. Both the carton and

the glass vial of rhC1INH are labelled as 'Ruconest® (C1 esterase inhibitor [recombinant]), powder for solution for injection. For intravenous infusion 2100U/vial'. One vial contains 2100 U of rhC1INH, corresponding to 2100 U/14 ml after reconstitution, or a concentration of 150 U/ml.

8.1.4 Storage Conditions

RhC1INH will be stored at 2-25ºC at the pharmacy of the study sites (shelf life 48 months when stored at 2-25°C, prior to reconstitution) in a dedicated, secure and temperature-monitored study room at the in-house pharmacy which will be inaccessible to unauthorized personnel. Drug accountability will be monitored according to ICH-GCP E6 (R2) Guideline for Good Clinical Practice. More details are described in the Study Pharmacy Manual.

8.2 Administration of Experimental and Control Interventions

8.2.1 Experimental Intervention

RhC1INH (Ruconest®) will be administered at (150 U/ml) at a 50 U/kg dose (max dose of 4200 U) as a slow intravenous injection via a peripheral or central intravenous line in approximately 5 minutes every 12 hours; for 4 days. A total of 8 doses will be administered. (The licensed dosage for rhC1INH is weight-based (50 U/kg up to 84 kg and 4200 U for a bodyweight of $\geq$84 kg). This is based on the aim to at least achieve a level of 0.7 U/ml C1-INH in patients with hereditary angioedema (lower limit of normal of C1-INH activity). Simulation studies have revealed that this aim is achievable with the licensed dosing[45].

However, these simulations have also shown that C1-INH levels will be lower when using 50 U/kg compared to 4200 U in patients with a bodyweight < 84kg. As for the current trial the aim is not to correct underlying *absolute* C1-INH deficiency (as in case of hereditary angioedema), but to ensure that a level of at least twice the serum concentration will be achieved in the vast majority of patients, we will use the licensed dose.

In patients with normal C1-INH levels, the chosen dose will increase plasma C1-inhibitor activities by at least 100% (4200 U) respectively. To maximize efficacy rhC1INH will be administered repeatedly over 4 days. Maximal volume of the injection is 28ml (4200 U) per administration.

Repeated administration of rhC1INH was chosen for several reasons. First, hyperinflammation caused by SARS-CoV-2 is a phenomenon that may last for several days, and hence sustained inhibition of the CS and the KK system is required. Second, the elimination half-life of rhC1INH was 2.5 hours[32]. Third, a decline of C-INH activity to pre-administration levels was demonstrated within four to six hours after administration of rhC1INH at a dose of 50 U/kg[46].

Duration of exposure to rhC1INH will be approximately 4 days. Participants will be followed in hospital for at least 12 hours after the last dose and via structured telephone interviews four weeks later.

8.2.2 Control Intervention

Participants in the control arm will receive SOC treatment according to local guidelines.

8.3 Dose / Device Modifications

The only modification will be omission of subsequent doses of rhC1INH if the participant develops a type 1 allergic reaction including an anaphylactic reaction after any dose of rhC1INH. If the patient is discharged or transferred to another facility the intervention will be ceased.

8.4 Compliance with Study Intervention

RhC1INH syringes for all administration time points on a given day will be prepared by the pharmacist. The study drug including the exact time for administration of the first and subsequent syringes will be entered into the electronic prescribing system/chart or added to the paper chart. As this study involves IV administration of the IMP by nurses, patient compliance measures are not necessary. IMP will be administered by a nurse according to the site's standard procedures. Two nurses will check the IMP with respect to dose, study number and patient's identity before administration to the patient. Nurses will document the syringe number and the time of administration in the electronic or paper chart.

8.5 Data Collection and Follow-up for Withdrawn Participants

Patients can leave the study at any time for any reason if they wish to do so without any consequences. The Investigator can decide to withdraw a patient from the study for medical reasons. A patient who experiences a treatment-emergent anaphylactic reaction will not be permitted to receive further treatment with rhC1INH.

Patients who withdraw informed consent will not be followed up. Although incomplete, data and samples collected up to the discontinuation will be analyzed. Personal data, samples and the confidentiality of these patients will be managed according to the same standards as per other evaluated completed patients.

Patients who are withdrawn from the study because they did not receive any study medication will not be followed up. Patients who are withdrawn because SARS-CoV-2 infection was not confirmed will be followed only for safety assessments.

Patients who are discontinued from the study because of a type I allergic reaction during the treatment phase are followed in the same fashion as planned for all participants.

8.6 Trial Specific Preventive Measures

All participants will receive standard supportive care established at the sites which may include off-label use of certain drugs that are deemed to be effective in COVID-19 (*e.g.* hydroxychloroquin, lopinavir/ritonavir). In addition, escalation of treatment with off-label use of certain drugs (*e.g.* tocilizumab) in deteriorating participants is allowed during the trial.

In terms of risk, rhC1INH, a recombinant human protein, has a very favorable side effect profile and major adverse events attributable to study medication are not expected in this study. Anaphylactic reactions are exceptionally rare and have only been observed in a single patient with a pre-existent allergy to rabbits. Although patients with rabbit allergy will be excluded from participating in the study, included subjects will be monitored for anaphylactic events during and for at least 12 hours after the last administration of rhC1INH. If patients develop type 1 allergic reactions following the administration of rhC1INH treatment according to in-house guidelines (potentially involving administration of antihistamines, corticosteroids, intravenous fluids and if needed adrenaline) will be provided.

No influence of rhC1INH on laboratory parameters or vital signs, including ECG was observed in previous clinical studies. Hence, we will not perform additional laboratory monitoring exceeding routine laboratory controls planned by the treating team (with the exception of the above-mentioned additional biomarker analyses). No interactions with other drugs are known.

If female, patients must be/have:

- Post-menopausal, defined as the absence of menses for at least one year, OR Surgically sterile, defined as a bilateral tubal ligation at least 6 months prior to administration of study drug, bilateral oophorectomy, or complete hysterectomy, OR

- Using an effective means of contraception with a failure rate less than 1% per year (*e.g*. oral contraceptives, contraceptive injections, intrauterine device, double-barrier method, male partner sterilization), OR
- A negative pregnancy test before study entry/randomization. We expect the rate of female patients of childbearing age who may become pregnant to be <10% of all patients potentially eligible for this study. Due to the short treatment period (4 days) and half-life of rhC1INH (2.5 hours) an effective means of contraception is not mandatory during the hospitalization treatment phase. At least one method of contraception (oral contraceptives, barrier methods, approved contraceptive implant or abstinence) is required for the entire follow up study period
- Concomitant Interventions (treatments)

All participants will receive standard supportive care established at the sites which may include off-label use of certain drugs that are deemed to be effective in COVID-19 (e.g. hydroxychloroquin, lopinavir/ritonavir, prophylactic anticoagulation). In addition, escalation of treatment with off-label use of certain drugs (. tocilizumab) in deteriorating participants is allowed during the trial. Randomization will be stratified by site to compensate for different standards of supportive care. Off-label use of drugs for the treatment of COVID-19 will be recorded in the CRF. Escalation of treatment is one outcome of interest.

Patients are allowed to use all their regular medication during the trial. In addition, patients will receive all drugs required for the treatment of COVID-19 or its consequences. The care of the participant will remain unaffected by inclusion into the study or by study group assignment.

8.7 Study Drug / Medical Device Accountability

The in-house Pharmacy will be responsible for maintaining accurate drug storage and dispensing logs including records about shipments to the trial sites, return to the Sponsor and document on-site destruction if applicable. Drugs will be stored in accordance with GCP and GMP requirements and will be inaccessible to unauthorized personnel. Lot/batch numbers will be recorded. All unused and used rhC1INH vials will be retained at the site until drug inventory has been completed by the Monitor.

The study staff will use study drugs only within the framework of this clinical study and in accordance with this protocol. Receipt, distribution, return, and destruction (if any) of study drugs will be properly documented. Accountability records will include dates, quantities, batch/serial numbers, expiration dates, and patient numbers and sign off by the pharmacist or nurse. These records will adequately document that the patients were provided the doses as specified in the protocol and should allow reconciliation activities from Monitor for all rhC1INH received from the Sponsor. The drug accountability process will be described in further detail separately in the Monitoring Manual.

8.8 Return or Destruction of Study Drug / Medical Device

Any study product that remains unused at the termination of the study will be returned to the manufacturer or destroyed in accordance with institutional policies and this process will be recorded.

9. STUDY ASSESSMENTS

9.1 Study Flow Chart(s) / Table of Study Procedures and Assessments

Study Periods	Screening	Intervention Period				Follow-up	
Visit	1	2	3	4	5	6	7
Time (weeks or days, or hours)	d-1[1]	d0	d1	d2	D3-13 during ad-mission	d14 (+/-2d) or dis-charge	3 months post treat-ment)[9]
In- /Exclusion Criteria	x						
Patient Information and Informed Consent	x						
Randomization		x					
Demographics	x						
Medical History	x						
Physical Examination	x	x	x	x	x	x	
Vital Signs including Respiratory Rate[7]		x	x	x	x	x	
Laboratory Tests (blood)[3]		x	x	x	x	x	
Virology testing		x[2]			x[2]	x[2]	
Administer Study Medication[4]		x	x	x	x		
Assessment of WHO ordinal scale		x	x	x	x	x	x
Assessment of ICU admission and/or mechanical ventilation		x	x	x	x	x	x
Measure Oxygenation levels[8]		x	x	x	x	x	
Monitoring QT intervals[10]		x	x	x	x	x	
Prior/Concomitant Medications	x	x	x	x	x	x	x
Laboratory assessment in detail:							
Routine full blood count[3]		x	x	x	x	x	
Routine coagulation studies[3]		x	x	x	x	x	
Routine biochemistry including LDH[3]		x	x	x	x	x	
Serum pregnancy test	x[5]						
Ferritin, IL-6, D-Dimer[3]		x	x	x	x	x	
C1INH concentration[6]		x	x	x	x	x	
C1INH activity[6]		x	x	x	x	x	
Complement proteins (e.g. C3, C4)[3]		x	x	x	x	x	
(Serious) Adverse Events	x	x	x	x	x	x	x

[1]Eligibility criteria to be assessed within 48 hours upon hospital admission.

[2]Inclusion of patients based on suggestive clinical symptoms and results from radiological studies will be possible. However, a nasopharyngeal swab for PCR validation of confirmed SARS-CoV-2 infection must be performed in all subjects no later after inclusion of the patients if not already performed before inclusion into the study. Subsequent virological testing for SARS-CoV-2 will be performed on day 4-7 (according to local standard) and again on day 14 if participants are still admitted.
[3]Routine laboratory tests will be performed once before 1st administration of study medication on (day 0 through day 3) and once daily during hospital admission according to standard procedures of the study site, but will include a set of hematology, coagulation and biochemistry parameters. Additional blood samples will be collected at the same time.
[4]IMP will be administered every 12 hours (+/-15 minutes) for 4 days. A total of 8 doses will be administered. Trial medication will be stopped in participants in whom PCR results are not consistent with confirmed COVID-19 and in patients that are discharged or transferred to another facility.
[5]A Serum hCG pregnancy test will be performed in women who may become pregnant, are sexually active, and do not use adequate contraceptive measures.
[6]Collection of samples will be once daily before 1st administration of study medication (day 0 through day 3) and once daily until hospital discharge.
[7]The worst value recorded on a given day will be documented.
[8]Oxygenation levels will be measured for patients on oxygen while hospitalized.
[9]3-months follow-up period begins post-treatment (Day 0). Assessments will be done daily until discharge and weekly via telemedicine interview or telephone call post-discharge.
[10]Monitoring of prolonged QT intervals in severely-ill subjects receiving multiple drugs.

Assessments of outcomes

9.1.1 Assessment of primary outcome

For the analysis of the primary endpoint, illness severity will be assessed on each day after enrollment (worst status) with the use of the WHO Ordinal Scale for Clinical Improvement and the score on day 7 will be analyzed stratified by its baseline value.

Patient State	Description	Score
Outpatient	No limitation in activities	1
	Limitation in activities	2
Hospitalized Mild disease	No oxygen therapy	3
	Oxygen by mask or nasal prongs	4
Hospitalized Severe disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation, mechanical ventilation +/- additional organ support	6
Death	Death	7

Figure 3: WHO ordinal scale for clinical improvement.
The worst score on a given day (between 0:00 and 24:00 hours) will be used for the categorization

Patient State	Description	Score
Outpatient	No limitation in activities	1
	Limitation in activities	2
Hospitalized Mild disease	No oxygen therapy	3
	Oxygen by mask or nasal prongs	4
Hospitalized Severe disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation, mechanical ventilation +/- additional organ support	6
Death	Death	7

9.1.2 Assessment of Secondary Outcomes

- For the analysis of time to clinical improvement of at least 2 points in the WHO Ordinal Scale for Clinical Improvement, clinical severity will be assessed every day until day 14 according to the Scale with the worst status for that day recorded.
- For the analysis of the proportion of patients alive and not having required invasive or non-invasive ventilation at 14 days after enrollment, admission to ICU with invasive or non-invasive ventilation or death will be assessed every day until day 14.
- For the analysis of the proportion of patients with ALI within 14 days after enrollment, PaO_2/FiO_2 will be determined daily until day 14. This is only relevant for patients with arterial blood gas sampling performed in the ICU or rarely in the medical unit.

9.1.3 Assessment of Other Outcomes of Interest

- The following assessments will be performed during the study: changes in WHO ordinal scale, ICU admission/discharge date, mechanical ventilation start/end date, admission/discharge date, all-cause mortality within 4 weeks.
- Supportive interventions provided to study subjects in addition to ventilatory support (e.g., the use of prone ventilation, high-frequency oscillatory ventilation (HFOV), paralytics, pulmonary vasodilators.
- The receipt of additional anti-inflammatory treatments such as tocilizumab will be recorded within 14 days.
- Vital signs will be recorded until discharge to determine the time to defervesence and clinical improvement.
- For the analysis of virological clearance of SARS-CoV-2, samples from the upper or lower respiratory tract will be collected at baseline (if not already performed on admission) to confirm SARS-CoV-2 infection and again on day 4-7 and day 14/discharge. Subsequently, samples will be analysed at the central laboratory of the study site using a quantitative assay according to validated standard in-house procedures. A negative result will be confirmed by a second test at least 12 hours apart.
- For the analysis in changes of biomarker levels CRP- LDH, D-dimer, IL-6 and lymphocytes will be measured in blood samples collected on day 0, 1-3 every day afterwards until day 14 or discharge. Blood samples will be sent to and analysed by validated methods in the central laboratory of respective study sites according to

standard in-house procedures.

- For the analysis of inflammatory proteins and cytokines blood samples will be collected on day 0, and daily until day 14 or discharge. For the analysis of rhC1INH pharmacokinetics (C1INH activity and concentration), blood samples will be collected at baseline, 5-10 minutes after the first administration and again immediately before and 5-10 minutes after a subsequent administration on day 1.

9.1.4 Assessment of Safety Outcomes

9.1.4.1 Adverse events

Participants will be followed for adverse events and outcomes for 3 months post treatment (Day 0). Assessments will be done daily while in the hospital and via a weekly follow-up telemedicine interview or telephone call after discharge.

Adverse events and clinical course including death within 4 weeks will be assessed by a weekly telemedicine or structured telephone interview for 3 months post-treatment. If necessary, medical charts will be reviewed, discharge summaries will be reviewed, or the patients' general practitioner will be contacted regarding specific follow-up information.
Every effort will be made to contact the trial subject and obtain complete data for follow-up. Conditions that started before signing informed consent will be recorded as medical history. Conditions that started or deteriorated after signing informed consent will be documented as AE. Definition of AE and SAE will be found in Section 10.

All AEs will be assessed and documented by the Investigators according to seriousness, intensity, causal relationship with study treatment, action taken with study treatment (*e.g.* withdrawal), specific treatment for AE and outcome. In addition, time of onset and AE duration will be recorded. Procedures are defined in Section 10. AEs will be recorded on the respective CRF pages. Participants will be asked about any new events/complaints during the hospitalization and up to 3 months post treatment. In addition, participants are asked to report any new events including hospital admission to the study team.

9.1.4.2 Laboratory parameters

Previous studies of rhC1INH have not shown any influence of rhC1INH on laboratory parameters. Hence, we will not perform additional laboratory monitoring exceeding routine laboratory controls planned by the treating team. If laboratory findings reveal abnormal values, they will be documented and reported as an AE if deemed clinically significant by the treating team or the Investigators.

9.1.4.3 Vital signs

No study specific vital signs will be assessed. All patients will be monitored regarding blood pressure, heart rate, respiratory rate and oxygen saturation as per standard operating procedures for the treatment of COVID-19 patients.

9.1.5 Assessments in Participants Who Prematurely Stop the Study

Patients who are withdrawn from the study will remain in the trial for the purpose of follow-up and data analysis and will be followed as planned with the following exceptions:

- Patients, who withdraw consent for all study measures, will be asked to have assessments performed as appropriate for the final 4 week study visit (*i.e*. a structured telephone interview). The same approach applies for all active study participants if the trial is stopped early. Patients who withdraw consent are at liberty to refuse any or all individual components of the final assessment.
- Patients who are withdrawn because they have not received any study medication will not be followed up and will not receive a final study assessment.
- Patients who discontinue the study because of safety concerns will be followed as planned.
- Patient with a type I allergic reaction will be referred to an allergist associated with the site for further work-up.

9.2 Procedures at Each Visit

9.2.1 Split into Subtitles by Type of Visit

Visit 1, Screening (Day -1)

Informed consent will be obtained before any study-specific tests or evaluations will be performed.

- Patients who are admitted to the hospital with confirmed SARS-CoV-2 infection will be screened. Screening will be based on routinely available data and laboratory tests with only the pregnancy test as study-specific intervention/test, if necessary, before screening of the patients. Results from prior tests that will be used for screening potentially eligible subjects will be recorded in the CRF.
- Selection of potential participants according to inclusion and exclusion criteria.
- Explanation of patient information including study purpose and signature of informed consent form
- Assessment of demographics, medical history, physical examination, and concomitant medication history
- Pregnancy test if applicable
- Eligibility check (in- and exclusion criteria)
- Study enrollment

Visit 2, Intervention period (Day 0)

- Randomization
- Assignment of the trial subject identification number
- Assessment of physical examination and concomitant medications
- Assessment of vital signs including respiratory rate.
- Assessment of WHO Ordinal Scale and need for oxygen supplementation
- Collection of blood (15ml) for analysis of ferritin, Il-6, D-Dimer, C1INH concentration, and collection of blood for the analysis of complement proteins (C3 & C4).
- Routine full blood count, coagulation and biochemistry studies will only be performed.
- Virology testing (SARS-CoV-2 PCR) on a nasopharyngeal sample if not already performed before inclusion into the study
- Following randomization, participants in the active treatment group will receive rhC1INH (150 U/ml) at a 50 U/kg dose (max dose of 4200 U) every 12 hours, as a slow intravenous injection in approximately 5 minutes. The time of dosing and the dose will be recorded.
- AEs occurring after the administration will be recorded.
- Routine laboratory tests will be performed daily during admission according to standard procedures, and should include a set of hematology, coagulation and biochemistry parameters. Additionally, blood (7ml) for analysis of ferritin, IL-6, D-Dimer, C1INH concentration will be collected at the same time point.
- Monitoring of prolonged QT intervals in severely-ill subjects receiving multiple drugs.
- Measurement of oxygenation levels.
- Monitor your heart rhythms in severely-ill subjects receiving multiple drugs
- Only in the active treatment group: Collection of blood (7ml) for analysis of C1INH levels and activity 10 (+/-5 minutes) after the first administration.

Visit 3, Intervention period (Day 1)

- Assessment of physical examination
- Administration of rhC1INH (150 U/ml) at a 50 U/kg dose (max dose of 4200 U) every 12 hours, in the active treatment group as per treatment schedule. The time of dosing and the dose will be recorded.
- Assessment of vital signs including respiratory rate.
- Assessment of WHO ordinal scale and need for oxygen supplementation

- Documentation of ICU admission and/or non-invasive or invasive ventilation
- Collection of blood (15ml) for analysis of ferritin, Il-6, D-Dimer, C1INH concentration, and collection of blood for the analysis of complement proteins (C3 & C4).
- Routine full blood count, coagulation and biochemistry studies will only be performed if results from prior tests on the same day are not available.
- Only in the active treatment group: Collection of blood (7ml) for analysis of C1INH levels and activity immediately before the first administration of rhC1INH on that day.
- Routine laboratory tests will be performed daily during admission according to standard procedures, and should include a set of hematology, coagulation and biochemistry parameters. Additionally, blood (7ml) for analysis of ferritin, IL-6, D-Dimer, C1INH concentration will be collected at the same time point.
- Monitoring of prolonged QT intervals in severely ill subjects receiving multiple drugs.
- Measurement of oxygenation levels.
- Monitor your heart rhythms in severely-ill subjects receiving multiple drugs
- Assessment of AEs and concomitant medications

Visit 4, Intervention period (Day 2)

- Assessment of physical examination
- Administration of rhC1INH (150 U/ml) at a 50 U/kg dose (max dose of 4200 U) every 12 hours in the active treatment group as per treatment schedule. The time of dosing and the dose will be recorded.
- Assessment of vital signs including respiratory rate.
- Assessment of WHO ordinal scale and need for oxygen supplementation
- Documentation of ICU admission and/or non-invasive or invasive ventilation
- Collection of blood (15ml) for analysis of ferritin, Il-6, D-Dimer, C1INH concentration, and collection of blood for the analysis of complement proteins (C3 & C4).
- Routine laboratory tests will be performed daily during admission according to standard procedures, and should include a set of hematology, coagulation and biochemistry parameters. Additionally, blood (7ml) for analysis of ferritin, IL-6, D-Dimer, C1INH concentration will be collected at the same time point.
- Monitoring of prolonged QT intervals in severely-ill subjects receiving multiple drugs.
- Measurement of oxygenation levels.
- Monitor your heart rhythms in severely-ill subjects receiving multiple drugs
- Assessment of AEs and concomitant medications

Visit 5, Intervention period (Day 3-13 during hospital admission)

- Assessment of physical examination
- Completion of treatment with rhC1INH (150 U/ml) at a 50 U/kg dose (max dose of 4200 U) every 12 hours (8 doses in total) in the active treatment group as per treatment schedule. The time of dosing and the dose will be recorded. (Treatment to be administer at Visit 5 on Day 3 ONLY)
- Assessment of vital signs including respiratory rate.
- Assessment of WHO ordinal scale and need for oxygen supplementation.
- Documentation of ICU admission and/or non-invasive or invasive ventilation
- Routine laboratory tests will be performed daily during admission according to standard procedures, and should include a set of hematology, coagulation and biochemistry parameters. Additionally, blood (7ml) for analysis of ferritin, IL-6, D-Dimer, C1INH concentration will be collected at the same time point. and collection of blood for the analysis of complement proteins (C3 & C4).
- Virological assessment (SARS-CoV-2 PCR in respiratory specimen) should be performed on day 4-7 (according to local standard).
- Monitoring of prolonged QT intervals in severely-ill subjects receiving multiple drugs.

- Measurement of oxygenation levels.
- Monitor your heart rhythms in severely-ill subjects receiving multiple drugs
- Assessment of AEs and concomitant medications

Visit 6, Follow-up (Day 14 (+/-2 days) or at discharge)

- Physical examination including body weight.
- Assessment of vital signs including respiratory rate.
- Assessment of WHO ordinal scale and need for oxygen supplementation
- Documentation of ICU admission and/or non-invasive or invasive ventilation
- Laboratory assessment including routine hematology, coagulation and biochemistry studies. Routine laboratory tests will be performed daily during admission according to standard procedures. Additional sample collection will be required in patients without routine testing on the day of discharge. Additionally, blood (7ml) for analysis of ferritin, IL-6, D-Dimer, C1INH concentration will be collected at the same time point, and collection of blood for the analysis of complement proteins (C3 & C4).
- Virological assessment (SARS-CoV-2 PCR in respiratory specimen) will be performed on day 14 (when still admitted) or at discharge.
- Monitoring of prolonged QT intervals in severely-ill subjects receiving multiple drugs.
- Measurement of oxygenation levels.
- Monitor your heart rhythms in severely-ill subjects receiving multiple drugs
- Assessment of AEs and concomitant medications

Visit 7, Follow-up (for up to 3 months, weekly telemedicine interview or telephone call)

- Assessment of adverse events, WHO ordinal scale, admission to ICU and/or mechanical ventilation by structured telemedicine interview or telephone call. If necessary, medical charts will be reviewed, discharge summaries will be obtained, or the patients' general practitioner will be contacted regarding specific follow-up information.

10. SAFETY

10.1 Drug Studies

During the entire duration of the study, all adverse events (AE) and all serious **adverse** events (SAEs) are collected, fully investigated and documented in source documents and case report forms (CRF). SAEs are to be documented on the Pharming SAE form and reported to PV within 24 hours of awareness. Study duration encompassed the time from when the participant signs the informed consent until the last protocol-specific procedure has been completed, including a safety follow-up period.

As the frequency of comorbidities in the patient population under study is significant and clinical complications are common in patients with COVID-19 infection, serious adverse events are possible in this setting. Hence, the Sponsor will establish periodic interim safety review meetings by an independent committee (see also Section 1.3).

10.1.1 Definition and Assessment of (Serious) Adverse Events and Other Safety Related Events

An **Adverse Event (AE)** is any untoward medical occurrence in a patient or a clinical investigation participant administered a pharmaceutical product, and which does not necessarily have a causal relationship with the study procedure. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. [ICH E6 1.2]. Any medical conditions

present before signing the informed consent will be reported in the medical history. Any AEs that occur between screening and first treatment are considered as Non-Treatment Emergent Adverse Events (TEAE).

A **Serious Adverse Event (SAE)** is classified as any untoward medical occurrence that:

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

In addition, important medical events that may not be immediately life-threatening or result in death, or require hospitalization, but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above should also usually be considered serious. [ICH E2A]. The EMA Important Medical Events (IME) list will be used as a reference for this.

An elective or pre-planned hospital admission will not be considered as a SAE.

Note: an AE or adverse drug reaction is considered “life-threatening” if, in view of either the Investigator or Sponsor, its occurrence places the patient at immediate risk of death. It does not include an event that, had it occurred in a more severe form, might have caused death. SAEs should be followed until resolution or stabilization. Participants with ongoing SAEs at study termination (including safety visit) will be further followed up until recovery or until stabilization of the disease after termination.

Regardless of the assumed causal relationship, all AE volunteered by the trial subjects or observed by the Investigator and/or his staff must be recorded on the AE forms. Records of AEs include patient data, description of the event, intensity, severity, relationship to the study drugs, temporal relation to the study drugs, action taken, description of outcome and documentation of the results of diagnostic and therapeutic measures.

Assessment of Causality
Both Investigator and Sponsor make a causality assessment of the event to the study drug, based on the criteria listed in the ICH E2A guidelines:

Relationship	Description
Definitely	Temporal relationship Improvement after dechallenge* Recurrence after rechallenge (or other proof of drug cause)
Probably	Temporal relationship Improvement after dechallenge No other cause evident
Possibly	Temporal relationship Other cause possible
Not related	Causal relationship can be ruled out
*Improvement after dechallenge only taken into consideration, if applicable to reaction	

Unexpected Adverse Drug Reaction
An “unexpected” adverse drug reaction is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator’s Brochure for drugs that are not yet approved and Product Information for approved drugs, respectively). [ICH E2A]

Suspected Unexpected Serious Adverse Reactions (SUSARs)
Adverse reactions are all untoward and unintended responses to an IMP related to any dose administered.

Unexpected adverse reactions are suspected unexpected serious adverse reactions (SUSARs) if the following 3 conditions are met:

1. The event must be serious.
2. There must be a certain degree of probability that the event is a harmful and an unintended reaction to the IMP, regardless of the administered dose.
3. The adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in the Investigator's Brochure.
   The Investigator and the Sponsor evaluate any SAE that has been reported regarding seriousness, causality and expectedness. If the event is assessed as related, either by the Investigator or the Sponsor, and is considered serious and unexpected, it is classified as a SUSAR.

Assessment of Severity
The Investigator will be required to assess the intensity of the AE and to record this assessment in the source documents. It is important to distinguish between serious criteria and severity of an AE. Severity is a measure of intensity whereas seriousness is determined by the criteria mentioned above. Investigators will use the following categories to quantify severity.

- Mild/grade 1: events require minimal or no treatment and do not interfere with the patient's daily activities.
- Moderate/grade 2: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe/grade 3: events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe adverse events are usually incapacitating.
- Life threatening/grade 4: potentially life-threatening or disabling. High-risk medical interventions.
- Fatal/grade 5: 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. AEs characterized as intermittent require documentation of onset and duration of each episode.

10.1.2 Reporting of Serious Adverse Events (SAE) and Other Safety Related Events

Reporting of SAEs
All SAEs must be reported immediately and within a maximum of 24 hours to the Sponsor via email at SafetyUS@pharming.com or fax at 1 201-389-8092.

Reporting of SUSARs
Fatal or life-threatening SUSARs need to be reported to the IRB and to Competent Authorities (e.g. FDA and EMA) within 7 calendar days. Follow-up information to Fatal or life-threatening SUSARs or other SUSARs need to be reported within 15 calendar days.

The Sponsor must inform all Investigators participating in the clinical study of the occurrence of a SUSAR.

Reporting and Handling of Pregnancies

In case a patient becomes pregnant during the study, the patient should be withdrawn immediately from the study. Any pregnancy during the treatment phase of the study and within 30 days after discontinuation of study medication will be reported to the Sponsor and within a maximum of 24 hours to the Sponsor. The course and outcome of the pregnancy should be followed up carefully, and any abnormal outcome regarding the mother or the child should be documented and reported to the Sponsor following the SAE reporting timelines.

Reporting of Special Situations
All special situations must be reported as an SAE within 24 hours of the Investigator's first knowledge of the event, even if considered non-serious and regardless or not of whether an (S)AE occurred.

Special situations may include, but are not limited to, the following:

- Use of a medicinal product during pregnancy or breastfeeding
- Use of a medicinal product in a pediatric or elderly population
- Reports of overdose, abuse, off-label use, misuse, medication error or occupational exposure
- Lack of therapeutic efficacy
- (Suspected) transmission of an infectious agent

Overdose
For this study any dose of rhC1INH greater than 100 U/kg will be considered an overdose.

Medication errors
A medication error is any unintentional error in the prescribing, dispensing, or administration of a medicinal product while in the control of the healthcare professional or patient. Examples of medication error include, but are not limited to: wrong dose, wrong or inadequate (route of) administration, equipment failure, preparation error. Medication errors are reported in the CRF. A description of the error must be provided and whether or not the medication error lead to any AE.

Periodic Reporting of Safety
In addition to the expedited reporting of SUSARs, the Sponsor will submit, once a year throughout the development program of the IMP, a developmental safety update report (DSUR) to the competent authorities (FDA and EMA) and IRBs, as appropriate.

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.

10.1.3 Follow up of (Serious) Adverse Events

Participants with SAEs (whether or not related to the study drug) will be monitored until resolution or until the event is considered chronic and/or stable by the Investigator and/or other physician who has the responsibility for the subject's medical care. Treating physicians including general practitioners will be contacted for follow-up results. If necessary, participants will be contacted directly for follow-up. Follow-up SAE reports will be reported according to the same timelines as initial reports. The outcome of AEs will be documented.

10.1.4 Ongoing Safety Monitoring and Stopping Rules

Adverse events and abnormal lab values will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. A subject who experiences a treatment-emergent anaphylactic/anaphylactoid reaction or thrombotic/thrombo-embolic event will not be permitted to receive further treatment with study drug until the review of the case, discussion with the treating Investigator and Pharming, and further evaluation as required is completed.

Further enrollment into the study, and further rhC1INH administration in all subjects, will be temporarily halted pending a review of the data if either of the following occur:
If one or more subjects experience a Grade 4 or Grade 5 adverse event possibly related to study drug administration, as assessed by the Investigator or Pharming, or
If two or more subjects experience a Grade 3 adverse event possibly related to study drug administration.

11. STATISTICAL METHODS

11.1 Hypothesis

Null hypothesis: Early administration of rhC1INH in addition to SOC is not associated with an improved clinical outcome in admitted COVID-19 patients as reflected by a lower score on the WHO Ordinal Scale on day 7 compared to SOC alone.

Alternative hypothesis: Early administration of rhC1INH in addition to SOC is associated with an improved clinical outcome in admitted COVID-19 patients as reflected by a lower score on the WHO Ordinal Scale on day 7 compared to SOC alone.

11.2 Determination of Sample Size

This is a pilot study investigating the efficacy and safety of rhC1INH in the prevention of clinical deterioration in a high-risk population with COVID-19. There is no reliable information available on the clinical evolution of the population included in the trial. Therefore, a precise quantitative pre-definition of the sample size of a population needed to obtain clinically significant primary endpoint is not possible at this point in time.

The assumptions which are proposed and the criteria to monitor and assess the primary endpoint are as follows:

The primary endpoint is a 7-point scale and the standard deviation σ can be expected as 1.5 points. A relevant effect δ is an advantage of at least 1 point. Then, the standardized difference is about $\delta/\sigma = 0.67$. For a fixed sample size design with a two-sided significance level of $\alpha = 0.05$ and a power of $1 - \beta = 0.80$, a sample size of $N = 2 \times 38$ is necessary. Because of a nonparametric test, we add 4 patients in each group, and because of drop-outs we add also 4 patients in each group to $N = 2 \times 46$. For a 2:1-randomization, a nonparametric analysis by the stratified Wilcoxon rank-test, and an adaptive group sequential analysis, the overall sample size is estimated as 120 = 80 + 40.
All patients are hospitalized, and it is very unlikely that the primary endpoint at Day 7 will not be measured. Nevertheless, a drop-out rate of about 10% is included.

Adaptive Design:

Two interim analyses after 32 and 80 patients are planned according to the Pocock adjusted levels $\alpha p = 0.0221$. Based on the results of an interim analysis the sample size can be adjusted (Lehmacher, Wassmer, 1999). There are no prespecified futility margins, but the DSMB can recommend stopping the study in the case of insufficient interim results.
The primary efficacy endpoint and the main secondary efficacy endpoints will be analyzed at latest at 14 days after start of treatment.

11.3 Statistical Criteria of Termination of Trial

There are no prespecified futility margins, but the DSMB can recommend stopping the study in the case of insufficient interim results.

11.4 Planned Analyses

11.4.1 Datasets to be Analysed, Analysis Populations

A review of the database will be conducted in a blinded manner shortly before the database will be locked, and any decisions made at that meeting (blinded Data Review Meeting) concerning the statistical analysis, *e.g.*, additional outcomes and populations, pooling of sites for efficacy analysis, will be documented in the Statistical Analysis Plan (SAP).

Missing data for the primary endpoint will be imputed by means of the Last-observation-carried-forward (LOCF) method, as sensitivity analyses some alternative imputation methods like imputation of the mean and multiple imputation will be additionally performed.

Safety Population: The Safety Population is defined as all patients who received at least one dose of rhC1INH. Statistical analyses will be based on the actual treatment the patient received.

Full Analysis Set/Intent-to-Treat Population: The FAS/ITT Population is defined as all patients who are randomly allocated to a study arm. Statistical analyses will be based on the treatment arm to which the patient was allocated. For quantitative data, the number of patients with non-missing information, means, medians, standard deviations and extremes will be determined. For qualitative data, absolute and relative frequencies will be calculated. Moreover, changes from baseline (V2, d0) will be calculated.

Per Protocol Population: The PP Population is defined as all patients in the FAS/ITT Population who complete the study and who do not have any major protocol violations, including the following:

- Patients who had major inclusion/exclusion criteria violations.

Sufficient compliance is expected because of IV administration of the IMP.
The PP Population will be determined by a blinded review in the Data Review Meeting (DRM) of the data prior to database lock. The primary efficacy analysis will be based on the FAS/ITT Population in this superiority trial; an additional efficacy analysis will be performed on the PP Population. The Safety Population will be used for all safety analyses.

11.4.2 Primary Analysis

The primary endpoint WHO Ordinal Scale measure at day 7 will be analyzed by means of the nonparametric Wilcoxon rank test stratified by its baseline values with two-sided α-level of 5%. Two adaptive interim analyses after 32 and 80 patients are planned according to the Pocock adjusted levels $\alpha_p = 0.0221$. The results of the sequential groups are combined by the inverse-normal-method (Lehmacher, Wassmer, 1999). There are no prespecified futility margins, but the DSMB can stop the study in the case of insufficient interim results.

Furthermore, 95% confidence intervals will be determined, and the results will be presented graphically by means of box-and-whisker plots.

The analyses will be carried out by the trial biostatistician.

11.4.3 Secondary Analyses

The secondary endpoints are time to improvement of at least 2 points on the WHO Ordinal Scale, the proportion of subjects with ARDS (defined by Berlin severity criteria) within 14 days after enrollment and the proportion of participants alive and not having required invasive or non-invasive ventilation at 14 days after enrollment. They will be tested only after a significant test of the primary endpoint (a priori ordered hypotheses), therefore, no alpha adjustment is necessary.

Quantitative secondary study parameters will be described based on their mean, standard deviation, median, IQR, minimums and maximums and portrayed by Kaplan-Meier plots and compared with the Wilcoxon rank test.

Qualitative secondary study parameters will be analyzed by means of absolute and relative frequencies. Pairwise Chi-Square tests will be carried out in order to compare each of the active treatments to placebo. Moreover, 95% confidence intervals for the treatment differences will be calculated.

Other outcomes of interest will be analyzed by means of descriptive and exploratory statistics.

The analyses will be carried out by the trial biostatistician.

11.4.4 Interim Analyses

Two interim analyses after 32 and 80 patients are planned according to the Pocock adjusted levels $\alpha_p = 0.0221$. Based on the results of an interim analysis the sample size can be adjusted (Lehmacher, Wassmer, 1999). There are no prespecified futility margins, but the DSMB can recommend stopping the study in the case of insufficient interim results.

11.4.5 Safety Analysis

The assessment of safety will be based mainly on the frequency of adverse events (AEs) that are treatment-emergent (TEAEs). Formal tests will not be conducted for differences in safety parameters between treatment groups.

The incidence of all treatment-emergent AEs will be tabulated after grouping by system organ class and preferred term. For each preferred term and summarized over each system organ class overall, the table will present the absolute number and proportion (%) of patients in each treatment group in whom the event occurred. The incidence of all suspected-IMP-related AEs will be tabulated similarly. The incidence of all treatment-emergent AEs will also be tabulated by severity categories.

Safety will also be summarized with respect to vital signs as mean levels by visit and change from baseline. Abnormal observations on physical exams will be listed.

11.4.6 Deviation(s) From the Original Statistical Plan

Deviations from the planned analyses will be discussed by the Sponsor and the biostatistician and consequently justified and reported in the final reports and the publications.

11.5 Handling of Missing Data and Drop-Outs

Withdrawn/discontinued participants will not be replaced. Missing data for the primary variable will be imputed by means of the Last-observation-carried forward (LOCF) method, as sensitivity analyses some alternative imputation methods like imputation of the mean and multiple imputation will be additionally performed.

12. QUALITY ASSURANCE AND CONTROL

The Sponsor and the local site Investigators are responsible for proper training of all involved study personnel and for implementing and maintaining quality assurance and quality controls systems with written SOPs and working instructions. All SOPs and working instructions will be prepared and critically evaluated by the Investigators and study nurses and monitors before commencement of the trial.

12.1 Data Handling and Record Keeping / Archiving

12.1.1 Case Report Forms

Study data will be recorded in a Case Report Form (CRF). A unique study code will be used for identification of participants in the CRF. Subjects will not be identified in the CRF by name or date of birth. Only authorized personnel will be able to make changes on the paper case report form (CRF) and are responsible for entering complete data. If source data is available as a print-out (*e.g.* laboratory values), this print-out will be kept on file in a source data folder, and the data necessary for the study is to be added to the CRF immediately.

12.1.2 Specification of Source Documents

Source data include the paper and electronic records of the hospitals at the respective study sites and all study documents (AE/SAE form, informed consent forms, laboratory reports etc.). Source data includes demographic data, visit dates, informed consent forms, randomization numbers, SAEs, AEs, concomitant medication, results of physical examination and information related to COVID-19 infection (*e.g.* data from viral sampling). Source data may be found either in the electronic or paper-based records of the hospitals at the respective study sites or as a print-out of study-related laboratory results which will be mailed to the local Investigators from the central laboratory hospitals at the respective study sites.

12.1.3 Record Keeping / Archiving

All study data will be archived for a minimum of 10 years after study termination or premature termination of the clinical trial.

12.1.4 Data Management System

The clinical trial data will be collected in a secure, validated EDC system.

12.1.5 Analysis and archiving

The EDC will be locked after all data was monitored and all raised queries have been resolved. Data is exported and transferred to the sponsor-Investigator by the CTU according to internally defined processes. Data will be archived by the by the Investigators as described above.

12.2 Monitoring

Monitoring will be carried out according to a predefined monitoring plan by Pharming (Sponsor), which is independent from the Investigators. Study documentation and all source data/documents will be accessible to monitors and all questions will be answered during inspections. Participants' data will be kept strictly confidential during the monitoring visits.

12.3 Audits and Inspections

Audits may be conducted by the IRBs and FDA and are independent from Investigators. Study documentation and all source data/documents will be accessible to auditors/inspectors and all questions will be answered during inspections. Participants' data will be kept strictly confidential during the monitoring visits. The Investigator should contact the Sponsor immediately if this occurs and must permit regulatory authority inspections.

12.4 Protocol Deviations

This study will be conducted as described in this protocol, except for an emergency situation in which the protection, safety, and well-being of the subject requires immediate intervention, based on the judgment of the Investigator (or a responsible, appropriately trained professional designated by the Investigator). In the event of a significant deviation from the protocol due to an emergency, accident, or mistake, the Investigator or designee must contact the Sponsor, or their agent, at the earliest possible time by telephone. This will allow an early joint decision

regarding the subject's continuation in the study. The Investigator and the Sponsor will document this decision. The IRB will be informed of all protocol changes by the Investigator in accordance with the IRB established procedure. No deviations from the protocol of any type will be made without complying with all the IRB established procedures.

Any protocol deviations that will affect the subject's safety or the study objectives may be considered Major Protocol Deviations upon review by the Sponsor. In addition, the following will be classified as Major Protocol Deviations:

- Inclusion/Exclusion Criteria
- Informed Consent not properly obtained
- Study Drug dosing variation
- SAE not reported in a timely manner

12.5 Confidentiality, Data Protection

All staff involved in the study is obliged to follow the ICH-GCP regulations, local requirements and privacy policy. Data will be archived. Any hard copies of the source documentation will be maintained in a locked filing cabinet with limited access. Subject confidentiality will be maintained throughout the clinical study in a way that ensures the information can always be tracked back to the source data. For this purpose, a unique subject identification code will be used that allows identification of all data reported for each subject.

Blood samples will be de-identified, labelled with a unique study code. Samples will be destroyed 10 years after study termination. Data and samples will be accessible only to authorized staff for scientific purposes. Throughout the study and during aforementioned inspections, strict confidentiality is guaranteed. In any publication and/or presentation, information will be provided in such a way that the participants cannot be identified, except with their permission and individual patient data will not be shown. Direct access to source documents will be permitted for the purpose of monitoring, audits and inspections by respective authorities.

12.6 Storage of Biological Material and Related Health Data

Blood and urine samples will be stored at -80°C in a dedicated freezer with limited access to unauthorized personal. The same applies to study data, which will be stored in a password secured database, and maintained by qualified IT specialists. All data including samples and source data (patients' charts) are stored for ten years.

13. PUBLICATION AND DISSEMINATION POLICY

Data derived from this clinical trial is considered to be the property of Pharming Technologies B.V. (the Sponsor). Study results will be presented at national and international conferences and published in peer reviewed medical journals. Trial results will be disseminated to the public and patients through publications in national and international journals, presentations at conferences and through publication of results in a public registry.

As a multicenter study, the first publication is intended to be a multicenter publication of the results of the overall study. In these cases, the Sponsor will be responsible for these activities and will work with the Investigators to determine how the manuscript is written and edited, the number and order of authors, the publishing journal/newsletter to which it will be submitted, and other related issues. The contributions of any contributor to the project will be taken into account in a fair and collegial way. Persons qualify for authorship if they have contributed significantly to the trial. The last author of the main publication will be the Principal Investigator, Dr. Bernstein. If a contributor will not qualify for authorship, her/his contribution will be mentioned as an acknowledgment.

Any individual publications, presentations or other disclosures of any study results by the centers or the local Investigator shall require the Sponsor's written consent and shall not occur until after the multicenter publication is published, which is intended to occur within 12 months after completion of the study at all study sites and lock of the database at all study sites.

All proposed publications are to be provided to Pharming a minimum of 60 days before submission to prospectively review any proposed publication, abstract, other type of disclosure that reports the results of the study. Pharming may wish to disclose results of the study after publication/presentation.

All financial support will be disclosed in any publication of study results.

In accordance with national and local requirements, this study will be listed in a publicly accessible clinical studies registry and be given a unique identifier (*e.g.* ClinicalTrials.gov). Additionally, the results of this study will be disclosed on a publicly accessible clinical studies results database, regardless of the outcome.

14. FUNDING AND SUPPORT

14.1 Funding

The trial will be conducted by Pharming Technologies B.V.(Sponsor). Pharming will provide IMP to each study site at no cost.

Patients may not be charged for participation or IMP.

15. INSURANCE

In the event of injury to a patient in the study as a result of the study, if the injury:

1. Results from a defect of the manufacturing of rhC1INH (Ruconest®), the patient injuries, to the extent compensable, will be compensated by Pharming or its insurance;
2. Results from the negligence of site personnel in administration of the study, the patient injuries, to the extent compensable, will be compensated by the site or its insurance;
3. Results otherwise from the conduct of the study, the patient injuries, to the extent compensable, will be compensated by the Sponsor or its insurance;

16. REFERENCES

1. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, Harcourt JL, Thornburg NJ, Gerber SI, Lloyd-Smith JO, de Wit E and Munster VJ. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *N Engl J Med*. 2020;382:1564-1567.
2. John Hopkins University & Medicine. Coronarvirus Resource Center. Coronarvirus COVID-19 Global Cases by the CSSE. Accessed on 14 April 2020.
3. Novel Coronavirus Pneumonia Emergency Response Epidemiology T. [The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China]. *Zhonghua Liu Xing Bing Xue Za Zhi*. 2020;41:145-151.
4. Siddiqi HK and Mehra MR. COVID-19 Illness in Native and Immunosuppressed States: A Clinical-Therapeutic Staging Proposal. *J Heart Lung Transplant*. 2020.
5. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S and Shang Y. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020.
6. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, Xie C, Ma K, Shang K, Wang W and Tian DS. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clin Infect Dis*. 2020.
7. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, Zhang Y, Song J, Wang S, Chao Y, Yang Z, Xu J, Zhou X, Chen D, Xiong W, Xu L, Zhou F, Jiang J, Bai C, Zheng J and Song Y. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med*. 2020.
8. Walport MJ. Complement. Second of two parts. *N Engl J Med*. 2001;344:1140-4.
9. Walport MJ. Complement. First of two parts. *N Engl J Med*. 2001;344:1058-66.
10. Thielens NM, Tacnet-Delorme P and Arlaud GJ. Interaction of C1q and mannan-binding lectin with viruses. *Immunobiology*. 2002;205:563-74.
11. Kase T, Suzuki Y, Kawai T, Sakamoto T, Ohtani K, Eda S, Maeda A, Okuno Y, Kurimura T and Wakamiya N. Human mannan-binding lectin inhibits the infection of influenza A virus without complement. *Immunology*. 1999;97:385-92.
12. Bibert S, Piret J, Quinodoz M, Collinet E, Zoete V, Michielin O, Menasria R, Meylan P, Bihl T, Erard V, Fellmann F, Rivolta C, Boivin G and Bochud PY. Herpes simplex encephalitis in adult patients with MASP-2 deficiency. *PLoS Pathog*. 2019;15:e1008168.
13. Schiela B, Bernklau S, Malekshahi Z, Deutschmann D, Koske I, Banki Z, Thielens NM, Wurzner R, Speth C, Weiss G, Stiasny K, Steinmann E and Stoiber H. Active Human Complement Reduces the Zika Virus Load via Formation of the Membrane-Attack Complex. *Frontiers in immunology*. 2018;9:2177.
14. Bermejo-Jambrina M, Eder J, Helgers LC, Hertoghs N, Nijmeijer BM, Stunnenberg M and Geijtenbeek TBH. C-Type Lectin Receptors in Antiviral Immunity and Viral Escape. *Frontiers in immunology*. 2018;9:590.

15. Gralinski LE, Sheahan TP, Morrison TE, Menachery VD, Jensen K, Leist SR, Whitmore A, Heise MT and Baric RS. Complement Activation Contributes to Severe Acute Respiratory Syndrome Coronavirus Pathogenesis. *mBio*. 2018;9.

16. Jiang Y, Zhao G, Song N, Li P, Chen Y, Guo Y, Li J, Du L, Jiang S, Guo R, Sun S and Zhou Y. Blockade of the C5a-C5aR axis alleviates lung damage in hDPP4-transgenic mice infected with MERS-CoV. *Emerg Microbes Infect*. 2018;7:77.

17. Sun S, Zhao G, Liu C, Fan W, Zhou X, Zeng L, Guo Y, Kou Z, Yu H, Li J, Wang R, Li Y, Schneider C, Habel M, Riedemann NC, Du L, Jiang S, Guo R and Zhou Y. Treatment with anti-C5a antibody improves the outcome of H7N9 virus infection in African green monkeys. *Clin Infect Dis*. 2015;60:586-95.

18. Sun S, Zhao G, Liu C, Wu X, Guo Y, Yu H, Song H, Du L, Jiang S, Guo R, Tomlinson S and Zhou Y. Inhibition of complement activation alleviates acute lung injury induced by highly pathogenic avian influenza H5N1 virus infection. *American journal of respiratory cell and molecular biology*. 2013;49:221-30.

19. Gao T, Hu M and Zhang X. Highly pathogenic coronavirus N protein aggravates lung injury by MASP-2-mediated complement over-activation. https://doi.org/10.1101/2020.03.29.20041962. 2020.

20. Heja D, Kocsis A, Dobo J, Szilagyi K, Szasz R, Zavodszky P, Pal G and Gal P. Revised mechanism of complement lectin-pathway activation revealing the role of serine protease MASP-1 as the exclusive activator of MASP-2. *Proceedings of the National Academy of Sciences of the United States of America*. 2012;109:10498-503.

21. Zhou Y, Lu K, Pfefferle S, Bertram S, Glowacka I, Drosten C, Pohlmann S and Simmons G. A single asparagine-linked glycosylation site of the severe acute respiratory syndrome coronavirus spike glycoprotein facilitates inhibition by mannose-binding lectin through multiple mechanisms. *J Virol*. 2010;84:8753-64.

22. Magro C, Mulvey JJ, Berlin D, Nuovo G, Salvatore S, Harp J, Baxter-Stoltzfus A and Laurence J. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: A report of five cases. *Transl Res*. 2020.

23. Sodhi CP, Wohlford-Lenane C, Yamaguchi Y, Prindle T, Fulton WB, Wang S, McCray PB, Jr., Chappell M, Hackam DJ and Jia H. Attenuation of pulmonary ACE2 activity impairs inactivation of des-Arg(9) bradykinin/BKB1R axis and facilitates LPS-induced neutrophil infiltration. *American journal of physiology Lung cellular and molecular physiology*. 2018;314:L17-L31.

24. Guy HR, Durell SR, Schoch C and Blumenthal R. Analyzing the fusion process of influenza hemagglutinin by mutagenesis and molecular modeling. *Biophys J*. 1992;62:95-7.

25. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C and Penninger JM. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med*. 2005;11:875-9.

26. Debreczeni ML, Nemeth Z, Kajdacsi E, Schwaner E, Mako V, Masszi A, Doleschall Z, Rigo J, Walter FR, Deli MA, Pal G, Dobo J, Gal P and Cervenak L. MASP-1 Increases Endothelial Permeability. *Frontiers in immunology*. 2019;10:991.

27. Dobo J, Major B, Kekesi KA, Szabo I, Megyeri M, Hajela K, Juhasz G, Zavodszky P and Gal P. Cleavage of kininogen and subsequent bradykinin release by the complement component: mannose-binding lectin-associated serine protease (MASP)-1. *PLoS One*. 2011;6:e20036.

28. Kaplan AP and Ghebrehiwet B. The plasma bradykinin-forming pathways and its interrelationships with complement. *Mol Immunol*. 2010;47:2161-9.

29. Nielsen EW, Waage C, Fure H, Brekke OL, Sfyroera G, Lambris JD and Mollnes TE. Effect of supraphysiologic levels of C1-inhibitor on the classical, lectin and alternative pathways of complement. *Molecular immunology*. 2007;44:1819-26.

30. Carugati A, Pappalardo E, Zingale LC and Cicardi M. C1-inhibitor deficiency and angioedema. *Molecular immunology*. 2001;38:161-73.

31. Hansen CB, Csuka D, Munthe-Fog L, Varga L, Farkas H, Hansen KM, Koch C, Skjodt K, Garred P and Skjoedt MO. The Levels of the Lectin Pathway Serine Protease MASP-1 and Its Complex Formation with C1 Inhibitor Are Linked to the Severity of Hereditary Angioedema. *Journal of immunology (Baltimore, Md : 1950)*. 2015;195:3596-604.

32. Davis B and Bernstein JA. Conestat alfa for the treatment of angioedema attacks. *Therapeutics and clinical risk management*. 2011;7:265-73.

33. van Veen HA, Koiter J, Vogelezang CJ, van Wessel N, van Dam T, Velterop I, van Houdt K, Kupers L, Horbach D, Salaheddine M, Nuijens JH and Mannesse ML. Characterization of recombinant human C1 inhibitor secreted in milk of transgenic rabbits. *J Biotechnol*. 2012;162:319-26.

34. Gesuete R, Storini C, Fantin A, Stravalaci M, Zanier ER, Orsini F, Vietsch H, Mannesse ML, Ziere B, Gobbi M and De Simoni MG. Recombinant C1 inhibitor in brain ischemic injury. *Annals of neurology*. 2009;66:332-42.

35. Igonin AA, Protsenko DN, Galstyan GM, Vlasenko AV, Khachatryan NN, Nekhaev IV, Shlyapnikov SA, Lazareva NB and Herscu P. C1-esterase inhibitor infusion increases survival rates for patients with sepsis*. *Critical care medicine*. 2012;40:770-7.

36. Caliezi C, Zeerleder S, Redondo M, Regli B, Rothen HU, Zurcher-Zenklusen R, Rieben R, Devay J, Hack CE, Lammle B and Wuillemin WA. C1-inhibitor in patients with severe sepsis and septic shock: beneficial effect on renal dysfunction. *Critical care medicine*. 2002;30:1722-8.

37. Hack CE, Ogilvie AC, Eisele B, Eerenberg AJ, Wagstaff J and Thijs LG. C1-inhibitor substitution therapy in septic shock and in the vascular leak syndrome induced by high doses of interleukin-2. *Intensive Care Med*. 1993;19 Suppl 1:S19-28.

38. Nurnberger W, Heying R, Burdach S and Gobel U. C1 esterase inhibitor concentrate for capillary leakage syndrome following bone marrow transplantation. *Ann Hematol*. 1997;75:95-101.

39. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W and Tan W. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 2020;395:565-574.

40. Jansen PM, Eisele B, de Jong IW, Chang A, Delvos U, Taylor FB, Jr. and Hack CE. Effect of C1 inhibitor on inflammatory and physiologic response patterns in primates suffering from lethal septic shock. *J Immunol*. 1998;160:475-84.

41. Dalle Lucca JJ, Li Y, Simovic M, Pusateri AE, Falabella M, Dubick MA and Tsokos GC. Effects of C1 inhibitor on tissue damage in a porcine model of controlled hemorrhage. *Shock*. 2012;38:82-91.

42. Panagiotou A, Trendelenburg M, Heijnen I, Moser S, Bonati LH, Breidthardt T, Fahrni G, Kaiser C, Jeger R and Osthoff M. A Randomized Trial of Recombinant Human C1-Esterase-Inhibitor in the Prevention of Contrast-Induced Kidney Injury. *JACC Cardiovasc Interv*. 2020.

43. Zeerleder S, Caliezi C, van Mierlo G, Eerenberg-Belmer A, Sulzer I, Hack CE and Wuillemin WA. Administration of C1 inhibitor reduces neutrophil activation in patients withsepsis. *Clin Diagn Lab Immunol*. 2003;10:529-35.

44. Nurnberger W, Petrik K, Burdach S and Gobel U. C1 esterase inhibitor (C1-INH) can reduce plasma concentrations of the complement activation product C5a. *Intensive Care Med*. 1994;20:242.

45. Farrell C, Hayes S, Relan A, van Amersfoort ES, Pijpstra R and Hack CE. Population pharmacokinetics of recombinant human C1 inhibitor in patients with hereditary angioedema. *British journal of clinical pharmacology*. 2013;76:897-907.

46. van Doorn MB, Burggraaf J, van Dam T, Eerenberg A, Levi M, Hack CE, Schoemaker RC, Cohen AF and Nuijens J. A phase I study of recombinant human C1 inhibitor in asymptomatic patients with hereditary angioedema. *J Allergy Clin Immunol*. 2005;116:876-83.

47. Riedl MA, Grivcheva-Panovska V, Moldovan D, Baker J, Yang WH, Giannetti BM, ReshefA, Andrejevic S, Lockey RF, Hakl R, Kivity S, Harper JR, Relan A and Cicardi M. Recombinant human C1 esterase inhibitor for prophylaxis of hereditary angio-oedema: a phase 2, multicentre, randomised, double-blind, placebo-controlled crossover trial. *Lancet*.2017;390:1595-1602.

48. Relan A, Bakhtiari K, van Amersfoort ES, Meijers JC and Hack CE. Recombinant C1-inhibitor: effects on coagulation and fibrinolysis in patients with hereditary angioedema. *BioDrugs*. 2012;26:43-52.

49. Nuijens JH, Eerenberg-Belmer AJ, Huijbregts CC, Schreuder WO, Felt-Bersma RJ, Abbink JJ, Thijs LG and Hack CE. Proteolytic inactivation of plasma C1- inhibitor in sepsis. *The Journal of clinical investigation*. 1989;84:443-50.

50. Fu Y, Cheng Y and Wu Y. Understanding SARS-CoV-2-Mediated Inflammatory Responses: From Mechanisms to Potential Therapeutic Tools. *Virol Sin*. 2020.