Statistical Analysis Plan

Recombinant human C1 esterase inhibitor (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).

(Study No.: C1 6201)

Protocol:

Version	Date	Description
3.0	12 January 2021	Final version

SAP:

Version	Date	Author	Description
1.0	01APR2021	Michael Bulitta	Initial Version

SAP APPROVAL

Reviewed and Agreed by:

SAP Author/Statistician:		SAP Reviewer:	
Dipl.-Stat. Michael Bulitta CRM Biometrics GmbH	Date	D. Schmitz, M. Sc. CRM Biometrics GmbH	Date

Reviewed and Approved by:

Clinical Trial Responsible:		Clinical Project Manager:	
Prof. Dr. Dr. Bruno Giannetti PharmingTechnologies B.V.	Date	Leisa Waynick PharmingTechnologies B.V.	Date

TABLE OF CONTENTS

Abbreviations

Abbreviation	Abbreviation in Full
AE	Adverse Event
ARDS	Acute respiratory distress syndrome
ATC	Anatomical, therapeutical, chemical
BL	Baseline
BP_d	Blood Pressure diastolic
bpm	Beats per minute
BP_s	Blood Pressure systolic
BRM	Blind Review Meeting
cGCP	Current Good Clinical Practice
CRF	Case Report/Record Form
CRP	C-reactive Protein
CSR	Clinical Study Report
COVID 19	Coronavirus disease 2019
d	Day
DD	Drug Dictionary
DMP	Data Management Plan
DRL	Drug Reference List
DRM	Data Review Meeting
DSMB	Data Safety Monitoring Board
ECMO	Extracorporeal Membrane Oxygenation
FAS	Full Analysis Set
hCG	Human Chorionic Gonadotropin
HCQ	Hydoxy Chloroquine
ICH	International Conference on Harmonization
ICTR	Integrated Clinical Trial Report
ICU	Intensive Care Unit

IL	Interleukin
IMP	Investigational Medicinal Product
IQR	Interquartile Range
ITT	Intention-to-treat
IV	Intravenous
LDH	Lactatedehydrogenase
LOCF	Last-observation-carried-forward
MedDRA	Medical dictionary for regulatory activities
PCR	Polymerase Chain Reaction
PP	Per Protocol
QC	Quality control
RRT	Renal Replacement Therapy
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard deviation
SOC	Standard of care
TEAE	Treatment-emergent Adverse Events
TFL	Tables/Figures/Listings
WHO	World Health Organization
wk	week

1 INTRODUCTION

General information on methods and procedures for the statistical evaluation are described in the Study Protocol, Final Version 3.0 dated 12 January 2021. The Statistical Analysis Plan (SAP) presented here outlines the evaluations to be performed in the final statistical analysis, including details with respect to endpoint derivation, applied statistical methods, and presentation of the results.

This Statistical Analysis Plan refers to the final version of the Study Protocol including all approved Amendments by the Version date of the SAP. The SAP will be finalized after the Blind Review of the data and contains a comprehensive and detailed elaboration of the statistical methodology section of the protocol.

2 DESIGN OF THE CLINICAL TRIAL

The study is a Phase IIa, randomized, open-label, multi-center, controlled (Standard of care/SOC), parallel group clinical trial of the efficacy and safety of a Ruconest® add-on treatment to SOC conducted in the United States.

It is planned to include a total of approximately 120 patients (80 in experimental and 40 in control group, allocation ratio 2:1).

3 TIME SCHEDULE

The study consists of 7 visits: screening visit (Visit 1, Day -1), and intervention period visits Visit 2 (Day 0), Visit 3 (Day 1), Visit 4 (Day 2), Visit 5 (Day 3-13 during hospital admission), and follow-up visits Visit 6 (Day 14 ± 2 days or discharge) and Visit 7 (Week 4 ± 3 months). A detailed schedule of assessments is presented in the Study Protocol, Final Version 3.0 dated 12 January, 2021 Chapter 9.1.

4 TRIAL OBJECTIVES

Overall Objective

The purpose of this study is to determine whether rhC1-INH 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.

Primary Objective

The primary objective of the study is to determine if adding 4 days of treatment with rhC1-INH to SOC treatment in adult participants admitted with non-critically ill 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.

Secondary Objectives

To evaluate the effect of rhC1-INH 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.

Safety Objectives

The study aims to assess the safety of rhC1-INH 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 four-week follow-up.

5 SUBJECTS ASSESSMENTS

5.1 Outcomes

5.1.1 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.

5.1.2 Secondary outcomes

The secondary endpoints will evaluate the effect of rhC1-INH 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 enrolment.
- Proportion of participants alive and not having required invasive or non-invasive ventilation at 14 days after enrolment.
- 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 enrolment.

5.1.3 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 four 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 rhC1-INH in COVID-19 patients will be characterized by measuring the concentration of rhC1-INH and the activity of C1-INH and inflammatory cytokines and proteins (such as C3, C4).

5.3 Safety variables

The study will evaluate the safety of rhC1-INH 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. Moreover, vital signs, physical examinations, and laboratory variables will be assessed for safety.

- Adverse events.
- Physical examination.

A brief, general physical examination will be performed at all visits. Examination will include: height, body weight, eyes, ears/nose/throat, cardiovascular, respiratory, gastrointestinal, genitourinary, musculoskeletal, neurological and psychiatric according to the state of the art.

- Vital signs.

Vital signs will be measured at Visit 2, 3, 4, 5, and 6:
- Heart rate
- Respiratory rate
- Temperature
- Blood pressure (BP_s, BP_d)
- SpO_2.

- Pregnancy.

A serum hCG pregnancy test at will be performed at screening in women who may become pregnant, are sexually active, and do not use adequate contraceptive measures.

5.4 Other variables

The following other variables are documented in this clinical trial:

- Demographics and baseline characteristics are to be collected on all patients including age (calculated from year of birth), gender, race, and ethnicity.

Relevant specific medical history will be captured especially the information highlighted below:

- Signs and symptoms (fever, cough, myalgia, headache, sore throat, loss of taste/smell, breathlessness, other).
- Past medical history (diabetes mellitus, hypertension, obesity, cardiovascular diseases, chronic renal diseases, COPD).
- Concomitant medications prior to admission.
- Off label drugs for Covid 19 (Lopinar/Ritonavir, Hydoxy Chloroquine (HCQ), Remdesivir, other).
- SARS-CoV-2 PCR.
- CT scan chest.

5.5 Treatment exposure and compliance

RhC1-INH (Ruconest®) will be supplied by the production company Pharming Technologies, B.V., the Netherlands. RhC1-INH 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. RhC1-INH is purified from the milk of transgenic rabbits, and supplied as a sterile, preservative-free, white/off-white lyophilized powder for reconstitution for injection. RhC1-INH contains less than 0.002% of rabbit-related impurities. One international unit (U) of rhC1-INH activity is defined as the equivalent of C1-INH activity present in 1 mL of pooled normal plasma. Each vial of RhC1-INH contains 2100 U of rhC1-INH, 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 rhC1-INH contains 150

U of rhC1-INH per 1 mL in a 20 mM sodium citrate buffer with a pH of 6.8. rhC1-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 rhC1-INH vials and prepare the study medication. RhC1-INH is for intravenous use only. The reconstituted solution is administered as a slow intravenous injection over approximately five minutes. Recommended doses of rhC1-INH for the treatment of an acute angioedema attack are 50 U/kg if body weight <84 kg and 4200 U if >84 kg. 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.
RhC1INH will be administered (150 U/ml) at a 50 U/kg dose (max dose of 4200 U) as a slow i.v. injection every 12 hours for 4 days: a total of 8 doses will be administered.

A market batch will be used for the study.

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

RhC1-INH 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.

5.6 Derived variables

For quantitative variables the absolute changes from baseline (Visit 2) will be determined.

5.7 Withdrawal/discontinuation

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 of the Clinical Study Protocol.

5.8 Schedule of assessments

The following table shows the study schedule of the clinical trial.

Table 1: Study schedule

Study Periods	Scree-ning	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 admission	d14 (±2 d) or discharge	wk+4 (+3 months)[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	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[7]	x[7]	x[7]	x[7]		
Complement proteins (e.g. C3, C4) [3]		x	x	x	x	x	
Blood volume (mL), total/study specific	0/0	15/5	15/15	15/5	15/5	15/15	
(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 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.
93-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.

6 SAMPLE SIZE

This is a pilot study investigating the efficacy and safety of rhC1-INH 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 40 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.

7 DATA ENTRY AND VALIDATION

The data entry of all records documented in the CRFs is carried out by two independent data entry persons and result in two different data sets, which will be compared. The double-entered CRF-database will be corrected until conformity is proved. Furthermore, the data will be analyzed for completeness and several plausibility checks will be carried out.

8 COMPUTER SYSTEMS, SOFTWARE, AND VALIDATION OF PROGRAMS

8.1 Hardware

Hardware used at CRMB is:

- Network servers:
  FSCTX300S4, Raid 5, 4 x 146 GB Sas HDs, 16 GB Ram
- Desktops (workstations):
  7 Desktops based on AMD and Intel
- Scanners:
  F-Secure Antivirus 7.0 for Windows Server
  ESET NOD 32 Antivirus Version 9 is used on the desktops
- Backup method:
  On every Friday a full backup with Acronis True Image Echo for Windows Server is performed (system and data). For the rest of the week a differential backup is created. The backups are stored on internal (RAID 5) and external hard discs. A complete backup can be reimported using Veem.

8.2 Software

Software used at CRMB is:

- DMSys from Sigmasoft, Version 5.1,
- SAS Version 9.4,
- Windows Server 2019 Network servers,
- Windows 10 on Workstations,
- Microsoft Office 365 for reports, tracking of CRFs.

The data management is performed using the validated Data Management System DMSys, Version 5.1, and the statistical analysis is performed using the validated statistical analysis program SAS, Version 9.4 under Windows 10.

8.3 Validation of programs

All SAS programs start with a header in which the following information is contained:

- Name of the program,
- author,
- date of creation,
- used analysis DATA sets.

All tables, graphics and listings contain in footer the name of the program with which they were created, and the date of creation.

All formats will be filed in a permanent format catalogue.

All SAS programs will be checked by a second SAS programmer responsible for the quality control (QC). He/she will also check all LOG files for ERROR messages and WARNINGS. ERRORs must not occur. WARNINGs must be checked as to whether they lead to wrong results.

Furthermore, the SAS programmer responsible for the QC will check the tables, figures, and listing for completeness and consistency. The review will be documented on the Program Validation Form.

The biometrician responsible for evaluation will make sure that the specifications of planned analysis have been correctly converted in SAS programs.

Analyses will be carried out anticipating SDTM datasets.

8.4 Restitution of programs

All SAS programs used in the analysis as well as the OUTPUT (listings, tables) will be stored, and archived together with the source code of the programs at CRM Biometrics GmbH.

9 CODING

Concomitant medications will be coded by means of the Drug Reference List/Drug Dictionary (WHO-DD). Adverse events and diseases will be coded using MedDRA, respectively.

10 DATA CHECKS

Before starting the statistical analysis, plausibility checks will be performed. A detailed list of edit checks can be found in the Data Management Plan (DMP, Appendix 5).

11 QUERIES

After performing the plausibility checks and the comparisons of the two data entry databases, the errors of the data entry will be corrected. Queries/Data Clarification Forms will be generated in the case of inconsistencies or missing data in the CRF. According to the resolved queries, changes of the data will be performed in the Master file, if necessary. In the case of remaining unsolved queries, the Principal Investigator, the responsible statistician, the medical advisor and the project manager will decide how to handle these data and all decisions taken will be documented in detail.

12 BLIND REVIEW

A blind data review meeting will take place before closing the database. A listing of all protocol deviations with the proposed classification into minor and major protocol deviations and a list of all data irregularities or unresolved queries and a proposal for how the data are to be handled in the analysis, if necessary, will be provided in the Blind Review Meeting (BRM). At least the responsible biometrician and the Clinical Project Manager will sign the Blind Review Meeting Minutes.

13 DATABASE CLOSURE

After all queries have been replied, the database master file will be corrected, if necessary, and the decisions regarding the handling of the unresolved queries will be made, and the database will be closed.

14 STATISTICAL EVALUATION

14.1 General

All statistical analyses will be performed in accordance with the ICH E9 "Statistical Principles for Clinical Trials" guideline.

14.2 Datasets to be analyzed, 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.

The following analysis populations will be used for analysis of study data:

Safety population: The Safety Population is defined as all patients who received at least one dose of rhC1-INH. Statistical analyses will be based on the actual treatment the patient received.

Full Analysis Set (FAS)/Intention to treat (ITT) 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 (PP) 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.

14.3 Patient Disposition and Characteristics

Demography and other baseline characteristics will be summarized descriptively for the FAS, PP, and Safety population (if different), overall and by treatment group. For quantitative data, means, medians, standard deviations and extremes will be determined. For qualitative data, absolute and relative frequencies will be calculated. Important baseline characteristics will be further summarized by sex, race, and age.

14.4 Treatments (study drug, rescue medication, other concomitant therapies, compliance)

RhC1-INH 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.

Use of concomitant therapies will be listed.

14.5 Efficacy

Continuous variables will be summarized using the mean, standard deviation, median, minimum, maximum values. Categorical variables will be summarized using the cell frequencies and percentage of patients in each category.

All statistical tests performed will be two-tailed with significance determined by reference to the 5 % significance level, unless otherwise stated. The null hypothesis at all times will be the equality of the treatments being compared.

Furthermore, the results will be presented graphically.

14.5.1 Primary efficacy variable

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 40 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.

14.5.2 Secondary efficacy variable

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.

14.6 Safety

The assessment of safety will be based mainly on the frequency of adverse events (AEs) that are treatment-emergent (TEAEs). Formal statistical 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 examinations will be listed.

14.6.1 Adverse events

Adverse events will be coded according to MedDRA. The assessment of Safety will be based mainly on the frequency of AEs that are treatment emergent (TEAEs). Formal statistical 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 examinations will be listed.

Summary statistics for the absolute vital sign value and the changes from baseline will be presented using n, mean, standard deviation, median, minimum, and maximum.

14.7 Multi-site study

Individual site results will be presented by means of descriptive statistical methods for the demographical and primary efficacy data.

14.8 Multiplicity

Pocock's group sequential approach is used and the significance levels in the interim analysis are adjusted for the primary variable accordingly ($\alpha_p = 0.0221$).

14.9 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.

14.10 Interim Analysis

Two interim analyses after 40 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.

14.11 Subject disposition

Subject disposition data (number of subjects screened, assigned to treatment, completing each assessment and the primary reason for withdrawal, number of each analysis population, subjects excluded from each analysis population) will be summarized overall for all subjects.

A by-subject listing will be provided showing all screened subjects who were not eligible to be assigned to treatment together with the reason for non-eligibility.

Number and percentage of subjects assigned to treatment who discontinued the study prematurely will be summarized for the overall population and stratified by reason for study discontinuation. Multiple reasons are possible.

A by-subject listing will be provided showing for all subjects assigned to treatment whether they completed study (yes/no), the reason(s) for study discontinuation (where applicable), and the dates of:

- the informed consent,
- the first application of study medication,
- the last application of study medication,
- discontinuation / withdrawal, where applicable.

14.12 Extent of exposure

For each subject, the individual study duration will be calculated in days as "$d_{lastVisit} - d_{Visit\ 1} + 1$", where $d_{lastVisit}$ is the date of last visit documented and $d_{Visit\ 1}$ is the date of Visit 1, respectively.

Exposure to IMP will be summarized descriptively as (1) number of applications made and (2) study duration (in days).

Summary statistics will be tabulated for the safety population.

14.13 Medical history

Diseases will be coded according to MedDRA. Details of the Medical History will be listed.

14.14 Previous and concomitant medications

Medications will be coded by means of DRL (WHO-DD).

A previous medication is a medication used only before the date of first dose of study medication (medication end date < date of first dose of study medication). All other medications are concomitant.

If medication start and/or stop dates are missing or partial, the dates will be compared as far as possible with the date of first dose of study medication. Medications will be assumed to be concomitant, unless there is clear evidence (through comparison of partial dates) to suggest that the medication started and stopped prior to the first dose of study medication. If there is clear evidence to suggest that the medication stopped prior to the first dose of study medication, the medication will be assumed to be previous.

Medications starting after the completion/withdrawal date will be listed but will not be classified or summarized.

Information on previous and/or concomitant medication will be listed with flags to differentiate previous and/or concomitant medications.

14.14 Deviations 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.

15 VISIT WINDOWS

All data will be organized and analyzed according to the scheduled visits outlined in the protocol.

16 LANGUAGE AND HEADINGS

All tables, figures and listings as well as the Integrated Clinical Trial Report (ICTR)/Clinical Study Report (CSR) will be produced in English language.

17 ARCHIVING

After the finalization of the analysis and the reporting, the following documents will be provided to Pharming Technologies B. V. for archiving purposes:

- the CRFs,
- the resolved queries,
- the Statistical Analysis Plan (pdf format),
- the Blind Review protocol (pdf format),
- database on CD (SAS files),

- the Clinical Study Report (CSR) (WORD 2010 format and pdf format).

18 LIST OF STAFF

A list of key study personnel can be found in the Data Management Plan (DMP, Appendix 1).

19 REFERENCES

[1] ICH E9. Statistical Principles for Clinical trials. Step 5, 1 Sep 1998, CPMP/ICH/363/96

[2] Lehmacher W, Wassmer G. Adaptive sample size calculations in group sequential trials. Biometrics 1999 Dec;55(4):1286-90. doi: 10.1111/j.0006-341x.1999.01286.x.PMID: 11315085