APL-D-003-20

A Phase 3, Multicentre, Randomised, Controlled Trial to Determine the Efficacy and Safety of Two Dose Levels of Plitidepsin Versus Control in Adult Patients Requiring Hospitalisation for Management of Moderate COVID-19 Infection

CLINICAL TRIAL PROTOCOL

INVESTIGATIONAL MEDICINAL PRODUCTS: Plitidepsin

Protocol No: APL-D-003-20

EudraCT No: 2020-005951-19

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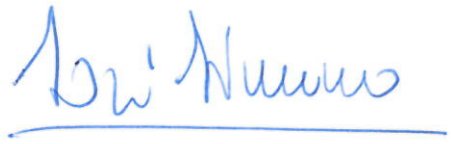
Sponsor:
Pharma Mar S.A.
Avda. De los Reyes 1
Poligono Industrial "La Mina"
28770 Colmenar Viejo
Madrid, Spain
Phone: + 34 91 846 6000
Fax: + 34 91 846 6003

Sponsor Signatory:
José Jimeno Doñaque, MD, PhD

SPONSOR APPROVAL
I have read the following and approve it:



30/03/2022

Jose M Jimeno, MD PhD
VP ; Global Head Virology & Inflamation

Date

SYNOPSIS

Title of study: A Phase 3, Multicentre, Randomised, Controlled Trial to Determine the Efficacy and Safety of Two Dose Levels of Plitidepsin *versus* Control in Adult Patients Requiring Hospitalisation for Management of Moderate COVID-19 Infection

Indication: Treatment of patients hospitalised for management of moderate coronavirus disease 2019 (COVID-19) infection

Number of investigators and study centres: Approximately 103 sites globally will be involved in this study.

Development phase: 3

Objectives and endpoints:

Objectives	**Endpoints**
Primary objective	
Efficacy primary objective	*Efficacy primary endpoint*
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control assessing the need of supplementary oxygen.	Time to sustained withdrawal of supplementary oxygen (11-category WHO Clinical Progression Scale ≤4) with no subsequent reutilisation during remaining study period (See Appendix 13 - World Health Organization (WHO) Clinical Progression Scale).
Key secondary objective	
Efficacy key secondary objective	*Efficacy key secondary endpoint*
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control assessing the time to sustained hospital discharge.	Time to sustained (i.e., with no subsequent readmission to Day 31) hospital discharge (since randomisation).
Secondary objectives	
Efficacy secondary objectives	*Efficacy secondary endpoints*
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control assessing clinical status by the 11-category WHO Clinical Progression Scale.	Clinical status by the 11-category WHO Clinical Progression Scale, at Day 8 (±1) (See Appendix 13 - World Health Organization (WHO) Clinical Progression Scale).
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg versus the control in terms of the need of advanced oxygen support.	Total duration of advanced oxygen support (high-flow nasal oxygen, extracorporeal membrane oxygenation - ECMO-, non-invasive ventilation or mechanical ventilation).
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of the need for intensive care support.	Percentage of patients in each study group requiring admission to ICU on Days 4, 8, 15 and 31.

Safety secondary objectives	*Safety secondary endpoints*
To compare safety/tolerability of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of adverse events, adverse reactions and mortality.	Frequency of: • Treatment-emergent adverse events (TEAEs); • TEAEs ≥ grade 3 according to the National Cancer Institute [NCI]-Common Terminology Criteria for AEs (CTCAE v.5.0); • Adverse events of special interest (AESIs); • Serious adverse events (SAEs); • Drug-releated serious adverse events (i.e., SARs) • Adverse events leading to treatment discontinuation; and • Deaths.
To compare safety/tolerability of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of abnormal laboratory parameters.	Change respect to baseline in individual study-defined laboratory parameters (See Section 7.2.4. and Appendix 2- clinical Laboratory Evaluations).
To compare safety/tolerability of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of variations of vital signs.	Change respect to baseline in individual vital signs (See Section 7.2.5.).
To compare safety/tolerability of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of ECG variations.	Change respect to baseline in individual ECG parameters (See Section 7.2.5.).
Other secondary objectives	
Other efficacy secondary objectives	*Other efficacy secondary endpoints*
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of hospital readmission related to COVID-19.	Percentage of patients in each study group who require hospital readmission related to COVID-19 through Day 31.
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of clinical evolution.	Percentage of patients in each study group and in each of the categories of the 11-point WHO Clinical Progression Scale on Days 4, 8, 15 and 31 (See Appendix 13 - World Health Organization (WHO) Clinical Progression Scale).
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of the need of supplementary oxygen.	Percentage of patients in each study group requiring oxygen therapy on Days 4, 8, 15 and 31.
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of the need of intensification of respiratory or intensive care support.	Time to intensification of respiratory support (WHO >6 [intubation]) (See Appendix 13 - World Health Organization (WHO) Clinical Progression Scale).
	Total duration of intensive care unit (ICU) stay for each study group.
	Percentage of patients in each study group requiring high-flow oxygen on Days 4, 8, 15, and 31.
	Percentage of patients in each study group requiring non-invasive mechanical ventilation on Days 4, 8, 15 and 31.

	Percentage of patient in each study group requiring invasive mechanical ventilation or ECMO on Days 4, 8, 15, and 31.
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of the need of intensification of pharmacological therapies.	Time to initiation with immune-modulating drugs. Time to initiation with antiviral drugs.
	Percentage of patients receiving subsequent immune-modulating drugs on Days 4, 8, 15 and 31. Percentage of patients receiving subsequent antiviral drugs on Days 4, 8, 15 and 31.
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of superinfections.	Percentage of patients in each study group with nosocomial infection by Days 4, 8, 15 and 31.
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of mortality.	Mortality in each study group on Days 4, 8, 15 and 31.
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of the evolution of viral load.	Change in the viral load of acute respiratory distress syndrome due to coronavirus 2 (SARS-CoV-2) in each study group from Day 1 before administration of the study drug until Day 8.
	Percentage of patients in each study group with undetectable viral load of SARS-CoV-2 on Day 8.
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of the evolution of inflammatory markers.	Change in inflammatory biomarkers (C-reactive protein [CRP], ferritin, interleukin [IL]-6, IL-1β, IL-10 and tumour necrosis factor alpha [TNFα]) in each study group from baseline until Days 2, 3, 4, 8, and 31.
To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of the immune response against SARS-CoV-2.	Change respect to baseline in individual serological assessments.
To compare efficacy in the primary endpoint and describe safety/tolerability of pooled plitidepsin arms *versus* control	Time to sustained withdrawal of supplementary oxygen (11-category WHO Clinical Progression Scale ≤4) with no subsequent reutilisation during remaining study period (See Appendix 13 - World Health Organization (WHO) Clinical Progression Scale).
To compare efficacy in the primary endpoint and describe safety/tolerability between plitidepsin arms (1.5 *versus* 2.5 mg) in case both are significantly superior to the control	Time to sustained withdrawal of supplementary oxygen (11-category WHO Clinical Progression Scale ≤4) with no subsequent reutilisation during remaining study period (See Appendix 13 - World Health Organization (WHO) Clinical Progression Scale).
To explore the influence of risk factors or scores for clinical deterioration that were not individually included	Obesity, hypertension, age and individual co-morbidities included in the Charlson Index (Appendix 11- Age-adjusted Charlson Index), ISARIC-4C score (Appendix 14 - ISARIC 4C Mortality & 4C Deterioration Scores), or vaccination status.
To compare the results obtained before and after the change of the	Time to sustained withdrawal of supplementary oxygen (11-category WHO Clinical Progression Scale ≤4) with

primary and key secondary endpoint (protocol v.6 *versus* v.7).	no subsequent reutilisation during remaining study period, before (protocol v.6) and after the amendment (protocol v.7).
	Time to sustained (i.e., with no subsequent readmission to Day 31) hospital discharge (since randomisation), before (protocol v.6) and after the amendment (protocol v.7).

Methodology/study design:

This is a multicentre, open-label, controlled Phase 3 study in which adults requiring hospital admission and O_2 supplementation for management of moderate COVID-19 infection will be randomised in 1:1:1 to:

- Plitidepsin 1.5 mg arm: Patients will receive plitidepsin 1.5 mg/day intravenous (IV) in addition to dexamethasone on days 1 to 3 (See Section 5. Study treatments).
- Plitidepsin 2.5 mg arm: Patients will receive plitidepsin 2.5 mg/day IV in addition to dexamethasone on days 1 to 3 (See Section 5. Study treatments).
- Control arm: Patients will receive dexamethasone IV on Days 1 to 3. Additionally, in accordance with local treatment guidelines, patients in this group may receive a regulatory-approved antiviral treatment (See Section 5. Study treatments).

Randomisation will be stratified for 3 factors:

a) Geographical Region (Europe *versus* Rest of the World);
b) Charlson Comorbidity Index (0-1 *versus* >1) (Appendix 11 - Age-adjusted Charlson Index); and
c) Barthel Index (≥90 *versus* <90) (Appendix 7 - Barthel Index for Functional Assessment).

From treatment initiation on Day 1, patients will be followed in the hospital for at least 4 days and then through Day 31 (±3 days) or resolution/stabilisation of treatment-related adverse events, treatment-emergent adverse events of special interest, and SAEs. Patients discharged from the hospital prior to Day 8 will return to an out-patient clinic for assessments on Days 8 (±1 day) and 31 (±3 days). On Day 15, patients will be followed up in remote or on-site visits (Appendix 5 – Schedule of Assessments).

An Independent Data Monitoring Committee (IDMC) will oversee study conduct (safety and primary endpoint), including analysis of summary safety data as per the trial requirements.

Number of patients:

A total sample size of 609 patients (203 in each arm) with 530 events necessary for the analysis of the primary endpoint has been calculated based on a one-sided type I error rate of 1.25% (α-0.0125) with at least 80% power (β=0.2) to detect a target hazard ratio (HR) of 1.4 in the time to sustained withdrawal of supplementary oxygen, which means a decrease in the median time to the event from 8 days (control arm) to 5.7 days (plitidepsin). The sample size is adjusted for the multiple comparisons of each plitidepsin group with control group by a Bonferroni adjustment, although other advanced methods for correction of multiplicity will be used for the main analysis that will increase the power of the tests (https://www.fda.gov/media/102657/download). At the final analysis, if the HR is 1.27 or greater, in favour of any plitidepsin arm (equivalent to a decrease in the median time to sustained withdrawal of supplementary oxygen of 1.7 days or greater), then it is expected that the null hypothesis (i.e., HR≤1) will be rejected.

A futility analysis for efficacy/safety will be performed when 33% of events (sustained withdrawal of supplementary oxygen) have been reached. The rho family of beta-spending functions (with ρ=1.18) will be used for the calculation of the stopping boundaries for futility in order to control type II error. The stopping boundaries for the futility analysis will be calculated with the actual

number of events available in the Intent-To-Treat (ITT) population at that moment; if the number of events at the futility analysis is 116 in a pair comparison, the beta-spending corrected p-value to reject the alternative hypothesis should be higher or equal than 0.4199 and this p-value is associated with a HR≤1.0382.

If the recommendation of the IDMC is to discontinue one of the plitidepsin dosing arms at this futility analysis following the stopping rules, the study will continue from that point on a 1:1 randomisation fashion until the completion of the remaining treatment arms (203 patients per arm and 353 events of sustained withdrawal of supplementary oxygen in total necessary to perform the final analysis). The patients still ongoing in the dropped arm would continue to be followed up as per protocol and a Bonferroni-adjusted type I error rate of 1.25% will be kept for the comparison of the primary endpoint between the remaining plitidepsin arm and the control arm.

Diagnosis and main criteria for inclusion and exclusion:

The following are the inclusion criteria:

1. Signed informed consent obtained prior to initiation of any study-specific procedures and study treatment.
2. Documented diagnosis of SARS-CoV-2 infection, determined by either qualitative polymerase chain reaction (PCR), antigen test by local laboratory, or any other validated method approved by the local health authority, from appropriate biological samples collected no more than 72 hours prior to study treatment on Day 1.
3. Patient meets category 5 on the 11-point WHO Clinical Progression Scale (Appendix 13 - World Health Organization (WHO) Clinical Progression Scale): requires hospitalisation and oxygen by mask or nasal prongs/cannula.
4. A maximum of 14 days from onset of COVID-19 symptoms to initiation of study treatment on Day 1.
5. Male or female aged ≥18 years.
6. Adequate bone marrow, liver, kidney, and metabolic function, defined by the following tests performed at local laboratory:
   - Absolute neutrophil count ≥500/mm^3 (0.5 x 10^9/L).
   - Platelet count ≥75,000/mm^3 (75 x 10^9/L).
   - Alanine transaminase (ALT), aspartate transaminase (AST) ≤3 x upper limit of normal (ULN).
   - Serum bilirubin ≤1 x ULN (or direct bilirubin <1 x ULN when total bilirubin is above ULN).
   - Calculated creatinine clearance ≥30 mL/min (Cockcroft-Gault equation).
   - Creatine phosphokinase (CPK) ≤2.5 x ULN except if the patient has had recent (i.e., in the last week) shivering episodes or trauma. In that case, the level of CPK should be ≤5 x ULN.
7. Agree not to participate in another interventional clinical trial through Day 31.
8. Females of reproductive capacity must have a negative serum or urine pregnancy test by local laboratory at study enrolment and must be non-lactating.
9. Females and males with partners of child-bearing potential must use effective contraception while on study treatment and for 6 months after last dose of plitidepsin. Patients in the control arm must use effective contraception during the time indicated in the approved product information (summary of product characteristics [SmPC] or leaflet). If no information is available in the approved product information, patients in the control arm must use effective contraception for at least one week after the study completion or the time indicated based on the investigator's discretion.

The following are the exclusion criteria:

1. Subjects with a pre-baseline (i.e., in the month preceding the current COVID-19 infection) impairment in general health condition for whatever reason except COVID-19, with a severe dependency for daily living activities (Barthel index ≤ 60/100, Appendix 7 - Barthel Index for Functional Assessment) or chronic oxygen therapy.
2. Having received treatment for COVID-19 in another clinical trial in the prior 4 weeks, except documented allocation in a placebo arm.
3. Evidence of respiratory failure at the time of randomisation, based on resource utilisation requiring at least one of the following: endotracheal intubation and mechanical ventilation, oxygen delivered by high-flow nasal cannula, non-invasive positive pressure ventilation, ECMO, or clinical diagnosis of respiratory failure (i.e., clinical need for one of the aforementioned therapies, which could not be administered in a resource-limited setting).
4. Patients with severe COVID-19, meeting score >5 on the 11-point WHO Clinical Progression Scale (Appendix 13 - World Health Organization (WHO) Clinical Progression Scale) or presenting, after an initial stabilisation prior to randomisation, any of clinical signs indicative of severe systemic illness, such as respiratory rate ≥30 per minute, heart rate ≥125 per minute, or PaO_2/FiO_2 <300. In case a direct measure of PaO2 has not been obtained, it should be imputed according to a referenced formula (Appendix 9 - Imputation of PaO_2 from SpO_2). For sites located over 1000 m above sea level, PaO2/FiO2 ratio will be adjusted (Appendix 10 - Adjustment of PaO_2 from a Site at High Altitude; see also Appendix 12 - Imputation of FiO_2 from oxygen flow values. High-flow oxygen therapy).
5. Patients receiving, at randomisation, treatment with antiviral therapy against SARS-CoV-2 or requiring anti-inflammatory/immunomodulating drugs beyond glucocorticoids with the exceptions listed below:
   - Prior administration of dexamethasone or equivalent glucocorticoid might be acceptable if:
     - The total daily dose is not higher than 10 mg of dexamethasone phosphate (equivalent to dexamethasone base 8.25 mg/day) or equivalent glucocorticoids (Appendix 15 – Glucocorticoid equivalent anti-inflammatory doses).
     - The duration of the treatment does not exceed 72 hours prior to study treatment Day 1.
   - Prior administration of an antiviral might be acceptable in the following circumstances:
     - For small molecules (e.g., remdesivir, molnupiravir, nirmaltrevir/ritonavir), they must have been given for an earlier stage of the disease, outside a clinical trial, and there should be a documentation of objective clinical deterioration plus evidence of persisting positivity for SARS-CoV-2 in appropriate biological samples. Last dose of previous antiviral drugs should have been administered at least 24 h before randomisation.
     - For antiviral monoclonal antibodies, they must have been given for an earlier stage of the disease (including pre-exposure prophylaxis), outside a clinical trial, and there should be a documentation of objective clinical deterioration plus evidence of persisting positivity for SARS-CoV-2 in appropriate biological samples. Last dose of antiviral monoclonal antibodies should have been administered at least 1 week before randomisation.
6. Patients receiving treatment with chloroquine or derivatives within 8 weeks before enrolment or during the study.

7. Patients receiving treatment with strong cytochrome P450 3A4 (CYP3A4) inhibitors or inducers (Appendix 4 – Inhibitors and Inducers of CYP3A4).
8. Viral illness (other than COVID-19) requiring therapy, except for patients with treated and adequately controlled (undetectable) human immunodeficiency virus infection.
9. Patients with uncontrolled known primary or secondary immunodeficiency, including chronic treatment with glucocorticoids (i.e., prednisone at a daily dose of >10 mg for >1 month, or another glucocorticoid at equipotent dose).
10. Any of the following cardiac conditions or risk factors:
    - Sinus bradycardia (<50 beats/min), sinus nodal dysfunction (sick sinus disease), atrioventricular block of any degree (PR >200 msec), or any other bradyarrhythmia (<50 beats/min), except for patients with permanent pacemakers;
    - Cardiac infarction, cardiac surgery or cardiac insufficiency episode within the last 6 months;
    - Known abnormal value of left ventricular ejection fraction (LVEF <low limit of normal (LLN)), unless documented confirmation of recovery (LVEF >LLN) in the previous month;
    - QT interval corrected using Fridericia's formula (QTcF) >450 msec for males or >470 msec for females;
    - History of known congenital or acquired QT prolongation;
    - Uncorrected hypokalaemia, hypocalcaemia (adjusted) and/or hypomagnesemia at screening;
    - Troponin test performed at local laboratory >1.5 x ULN; or
    - Need for an unreplaceable drug that prolongs QT and it is clearly associated with a known risk for torsades de pointes (TdP) (category 1 of Appendix 8a – Lists of Drugs That Prolong the QT Interval and/or Induce Torsades de Pointes Ventricular Arrhythmia); in case of being already on treatment with these aforementioned drugs, a minimum of 4 half-lives of the drug is required before replacement (if feasible).
11. Hypersensitivity to the active ingredient or any of the excipients (mannitol, macrogolglycerol hydroxystearate, and ethanol) or patients for whom dexamethasone, antihistamine H1/H2 or antiserotoninergic agents are contraindicated.
12. Females who are pregnant (negative serum or urine pregnancy test required for all females of child-bearing potential at screening) or breast feeding.
13. Females and males with partners of child-bearing potential (females who are not surgically sterile or postmenopausal defined as amenorrhea for >12 months) who are not using at least 1 protocol-specified method of contraception.
14. Any other clinically significant medical condition (including major surgery within the last 3 weeks before screening) or laboratory abnormality that, in the opinion of the investigator, would jeopardise the safety of the patient or potentially impact on patient compliance or the safety/efficacy observations in the study.

Test products, dose, and mode of administration:

Plitidepsin for injection is provided in vials containing 2 mg plitidepsin powder. For administration, vial contents are reconstituted by addition of 4 mL of solvent for plitidepsin to obtain a slightly yellowish solution containing 0.5 mg/mL plitidepsin with mannitol, macrogolglycerol hydroxystearate, and ethanol excipients. The required amount of plitidepsin-reconstituted solution is added to a bag containing 0.9% sodium chloride or 5% glucose for IV injection and administered as an IV infusion over 60 minutes.

For prevention of plitidepsin-related infusion reactions, administration of the following premedications should be ideally completed 20 to 30 minutes before initiating the plitidepsin infusion and, exceptionally, up to 2 h before plitidepsin infusion start:

- Palonosetron 0.25 mg IV (tropisetron 5 mg IV could be considered if palonosetron is not available)
- Diphenhydramine hydrochloride 25 mg IV (or equivalent, such as dexchlorpheniramine maleate 5 mg)
- Ranitidine 50 mg IV (or equivalent, such as famotidine 20 mg IV)
- Dexamethasone phosphate 8 mg IV (equivalent to 6.6 mg of dexamethasone base)

Additionally, on Days 4 and 5 patients treated with plitidepsin must receive tropisetron 5 mg PO/IV if tropisetron 5 mg IV was administered on Days 1, 2, and 3.

Reference therapy, dose, dose form, and mode of administration:

Dexamethasone: Patients on both plitidepsin and control arms will receive dexamethasone phosphate 8 mg/day (equivalent to 6.6 mg of dexamethasone base) IV on Days 1 to 3 (administered as a premedication in plitidepsin arms), followed by dexamethasone phosphate 7.2 mg/day (equivalent to 6 mg of dexamethasone base) PO/IV from Day 4 and up to a total cumulative dose of 60 mg of dexamethasone base (as per physician judgement according to patient clinical condition and evolution).

Remdesivir: Consistent with local treatment guidelines, patients randomised to the control arm may receive remdesivir 200 mg IV on Day 1 followed by 100 mg/day IV on Days 2 to 5.

Favipiravir: Consistent with local treatment guidelines, patients randomised to the control arm may receive favipiravir 1600 mg BID PO on Day 1, followed by 600 mg BID PO daily for 2 to 5 days.

Best Supportive Care (BSC): BSC consistent with National Institute of Health COVID-19 treatment guidelines (www.covid19treatmentguidelines.nih.gov) or local country guidelines will be provided to all study participants.

Duration of patient participation in study:

The study is expected to randomise approximately 609 patients (203 in each arm) over a period of 20 months.

Patients will be considered to be on study from signing the informed consent form until the end of the follow-up period. All treated patients will be required to complete follow-up through Day 31 (±3 days) or until resolution/stabilisation of any treatment-related adverse events, treatment-emergent adverse events of special interest, or SAEs that were initiated before Day 31.

Study populations:

Intent-to-Treat (ITT) Population: All patients randomised in the trial, regardless of whether they received treatment.

Full Analysis Set (FAS) Population: All randomised patients who have taken at least 1 dose of study treatment (plitidepsin or control) and with at least 1 postbaseline clinical status collected. FAS population will be analysed according to their randomised treatment.

Per Protocol Population: A subgroup of the FAS population that includes all patients who do not have important protocol violations that would interfere with the collection or interpretation of the efficacy data. Important protocol violations will be defined in the statistical analysis plan (SAP). Per Protocol population will be analysed according to their randomised treatment. Supportive efficacy analyses will be also performed on this population.

As Treated Population: All patients who received any exposure to study treatment (plitidepsin or control). As Treated population will be analysed according to the treatment they actually received.

Evaluation: Assessments for efficacy and safety (See Appendix 5 – Schedule of Assessments)

- Medical history at screening.
- Vital signs: temperature, sitting blood pressure, heart rate, respiratory rate, PaO_2 (obtained either by arterial blood gas analyses or from saturation of oxygen (SpO_2) by pulse oximetry), and its respective FiO_2 (Appendix 12 - Imputation of FiO_2 from oxygen flow values. High-flow oxygen therapy) to be measured at screening, on Days 1 to 3 just before start and end of each plitidepsin infusion (plitidepsin groups)/once daily on Days 1 to 3 (control group), further once daily while the patient is hospitalised, and on Days 8 (±1 day) and 31 (±3 days). To estimate the PaO_2/FiO_2 ratio, the required equation would be used for imputing PaO_2 from SpO2 (Appendix 9 – Imputation of PaO_2 from SpO_2), and the information on the oxygen delivery devices and oxygen flow prescribed would be utilised for imputation of FiO_2.
- Physical examination will be performed at Day 1 prior to initiation of study treatment, once daily during hospitalisation, and at follow-up visits on Days 8 (±1 day) and 31 (±3 days).
- Electrocardiogram: QTcF prolongation will be assessed at screening and on Days 1, 3 (postinfusion), and 31 (±3 days), and any QTcF values >500 msec will be flagged as increasing the likelihood of the treatment being proarrhythmic (per ICH Guidance E14).
- Haematology* (Appendix 2- Clinical Laboratory Evaluations): red blood cells (RBC), haemoglobin, haematocrit, white blood cells (WBC) with differential and platelet count will be measured at Day 1 prior to initiation of study treatment, once daily during hospitalisation, and at follow-up visits on Days 8 (±1 day) and 31 (±3 days). Testing to be performed by the local laboratory for screening and by central laboratory for on-study tests (tests performed within 24 hours of screening do not have to be repeated).
- Serum chemistry* (Appendix 2- Clinical Laboratory Evaluations): it will be performed at Day 1 prior to initiation of study treatment, once daily during hospitalisation, and at follow-up visits on Days 8 (±1 day) and 31 (±3 days), except, troponin T (high sensitivity), and NT-pro-BNP that will be measured on Day 1, 8 (±1 day) and 31 (±3 days). Tests performed within 24 hours of screening do not have to be repeated.
    - *Prestudy screening assessments:* ALT, AST, total bilirubin, sodium, potassium, calcium (albumin adjusted), magnesium, creatinine, calculated creatinine clearance (Cockcroft-Gault equation), CPK, and troponin (I or T according to local practice). Testing to be performed by the local laboratory.
    - *On study assessments:* ALT, AST, alkaline phosphatase, GGT, LDH, total bilirubin, direct bilirubin, glucose (fasting), sodium, potassium, calcium (albumin adjusted calculation), magnesium, blood urea nitrogen, creatinine, calculated creatinine clearance (Cockcroft-Gault equation), CPK, albumin, amylase, lipase, procalcitonin, troponin T (high sensitivity), and NT-pro-BNP. Testing to be performed by central laboratory.
- Coagulation (Appendix 2- Clinical Laboratory Evaluations): D-dimer will be measured during hospitalisation, and at follow-up visits on Days 8 (±1 day) and 31 (±3 days).
- Serological test: IgGs anti-SARS-CoV-2 will be measured on Day 1 and Day 31 (±3 days).
- Immunology: proinflamatory biomarkers **(**CRP, ferritin, IL1β, IL6, IL10, TNFα) will be measured in each study arm from baseline (Day 1 prior to start of the study treatment) to Day 4 and Days 8 (±1 day) and 31 (±3 days).
- COVID-19 viral load: quantitative real-time-reverse transcription polymerase chain reaction test (qRT-PCR) will be performed by central laboratory from oro-nasopharyngeal exudates on Day 1 prior to initiating treatment and on Day 8.
- Chest imaging: computed tomography (CT) scan or X-ray will be performed on Day 1, 8 (±1 day) and 31 (±3 days). Chest imaging performed within 48 hours of Day 1 is accepted

and does not have to be repeated. Images will be reviewed for the presence of pulmonary infiltrates, pleural fluid, atelectasis, pulmonary oedema, and other findings.

- Clinical status (including the need for oxygen supplementation): assessed using the 11-point WHO Clinical Progression Scale (Appendix 13 – World Health Organization (WHO) Clinical Progression Scale) will be performed, at least once a day, or whenever it changes, at screening, daily from Day 1 while hospitalised and on Days 8 (±1 day), 15 (±1 day), and 31 (±3 days).
- Functional status: assessed by the Barthel index score (Appendix 7 – Barthel Index for Functional Assessment); the pre-baseline (i.e., > 1 month prior to screening) should be recorded, in addition to Day 1 prior to initiating study treatment, at hospital discharge, and on Days 8 (±1 day), 15 (±1 day), and 31 (±3 days).
- All adverse events (AEs) and adverse reactions (ARs) will be measured daily until Day 31 (±3 days), except treatment-related adverse events, treatment-emergent adverse events of special interest, and SAES that will measured daily until resolution or stabilisation to at least Grade 1, or to an acceptable level according to the investigator and the sponsor of his/her designated representative. All AEs will be recorded and graded according to NCI-CTCAE version 5.0.

  The following AEs will be considered and monitored as AEs of special interest: musculoskeletal disorders, CPK increases, and rhabdomyolysis; hypersensitivity reactions, cardiac events and transaminase elevations, and hepatobiliary disorders.

- Concomitant medications: concomitant medications and treatments will be recorded for all patients from 28 days before the start of study treatment and through study Day 31 (±3 days). All COVID-19 vaccinations should be recorded, regardless of when they were given, until the end of study.

* Laboratory abnormalities will be recorded and graded according to NCI-CTCAE version 5.0. Laboratory abnormalities for which NCI-CTCAE grading is not available will be classified as the fold below the lower limit of normal (<LLN) or fold above the ULN.

Additionally, a dedicated QTc substudy will be performed in a subset of approximately 50 patients with ECGs collected over Days 1 to 3 using Holter monitors to assess treatment impact on QTc prolongation. See Appendix 8 (Protocol for QTc Substudy) for objectives and respective endpoints planned for the QTc substudy. The substudy patients will have blood sampling for pharmacokinetic assessments over Days 1 to 4.

Statistical methods:

Demographic and baseline disease characteristic data will be summarised descriptively. All summary tables for quantitative parameters will display n, mean, standard deviation, median, interquartile range (25^{th} and 75^{th} percentiles) and range (minimum and maximum). All summary tables for qualitative parameters will display counts and percentages. The use of imputation methods in visits of patients with missing values will be detailed in the SAP. The study treatment exposure will be assessed by the duration of treatment exposure during the study.

The primary endpoint, key secondary and other time-to-event efficacy endpoints will be evaluated in the ITT population. Other secondary efficacy analyses will be performed on the FAS population. Supportive analyses will be performed on the Per Protocol population. A sensitivity analysis will be performed for the primary endpoint and other time to event efficacy endpoints in the FAS population. The primary study analysis will be performed to demonstrate an effect on the primary endpoint. It will be calculated by means of the stratified log-rank test selecting the randomisation values of the stratification factors. The randomisation stratification factors are Geographical Region (Europe *versus* Rest of the World), Charlson Comorbidity Index (0-1 *versus* >1) (Appendix 11 – Age-adjusted Charlson Index) and Barthel index (≥90 *versus* <90) (Appendix 7 - Barthel Index for Functional Assessment). An unstratified log-rank test will also be calculated as a sensitivity analysis. A stratified Cox regression, with treatment as the single covariate, will be used to assess the magnitude of the treatment effect (i.e., the hazard ratio, HR) between the treatment arms comparisons. Cox regression will also be used to evaluate the influence of the stratification variables and other potential prognostic factors on the time-to-event efficacy endpoints. Time-to-event variables and their set time estimates will be analysed according to the Kaplan-Meier method. The main analysis will be adjusted for multiplicity using the Hochberg step-up procedure to preserve the type I error rate in the comparison of each dose of plitidepsin *versus* control arm. A futility analysis for efficacy/safety will be performed when 33% of events have been reached. The rho family of beta-spending functions (with ρ=1.18) will be used for the calculation of the stopping boundaries for futility in order to control type II error. If the accrual in any of the experimental arms is stopped after the interim futility analysis, the final analysis for the primary endpoint in the remaining arms will be performed at the 1.25% level using a Bonferroni-adjusted type I error rate.

Safety analyses will be based on the As Treated Population. All safety parameters will be summarised and also listed by treatment group and patient.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
ALT	alanine transaminase
AST	aspartate transaminase
BID	twice daily
BUN	blood urea nitrogen
CBC	complete blood count
CD	cluster of differentiation
CI	confidence interval
COVID-19	coronavirus disease 2019
CPK	creatine phosphokinase
CRO	contract research organisation
CRP	C-reactive protein
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CYP	cytochrome P450
CYP3A4	cytochrome P450 3A4
EC	Ethics Committee
ECG	electrocardiogram
ECMO	extracorporeal membrane oxygenation
eCRF	electronic case report form
eEF1A	eukaryotic elongation factor 1A
ERT	emergency response team
EUA	Emergency Use Authorisation
FAS	Full Analysis Set
FDA	Food and Drug Administration
FiO_2	fraction of inspired oxygen
$f_{u,human}$	unbound fraction in human plasma
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GTP	guanosine triphosphate
hACE2	human angiotensin converting enzyme 2
IB	investigator's brochure
IC_{50}	half-maximal inhibitory concentration
IC_{90}	90% maximal inhibitory concentration
ICF	informed consent form
$ICF_{total,\ in\ vitro}$	concentration used in the *in vitro* experiment
$ICF_{total,\ plasma}$	total target plasma concentration
ICH	International Conference on Harmonisation
ICU	intensive care unit
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
Ig	immunoglobulin
IL	interleukin
IMP	investigational medicinal product
IRB	Institutional Review Board
ITT	Intent-to-Treat
IV	intravenous

Abbreviation	Definition
IWRS	interactive web response system
LBR	lung-to-blood ratio
LBR_{rat}	lung-blood ratio in the distribution study in rats
LDH	lactate dehydrogenase
LVEF	left ventricular ejection fraction
MedDRA	Medical Dictionary for Regulatory Activities
MERS-CoV	Middle East Respiratory Syndrome coronavirus
NCI	National Cancer Institute
NF-κB	nuclear factor kappa B
NIV	non-invasive ventilation
NSCLC	non-small cell lung cancer
NT-pro-BNP	N-terminal pro-B-type natriuretic peptide
PaO_2/FiO_2	partial pressure of oxygen/fraction of inspired oxygen
PCR	polymerase chain reaction
PK	pharmacokinetic(s)
PO	oral administration
Q4W	every 4 weeks
qPCR	quantitative polymerase chain reaction
QTc	corrected QT interval
QTcF	QT interval corrected using Fridericia's formula
RBC	red blood cell
RNA	ribonucleic acid
RSI	reference safety information
RT-PCR	reverse transcription polymerase chain reaction
SAE	serious adverse event
SAP	statistical analysis plan
SAR	serious adverse reaction (i.e., drug-related serious adverse event)
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SmPC	summary of product characteristics
SOI	start of infusion
SpO_2	saturation of oxygen
TEAE	treatment-emergent adverse event
TNFα	tumour necrosis factor alpha
ULN	upper limit of normal
US	United States
WHO	World Health Organization

1. INTRODUCTION

Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses ranging from 60 to 140 nm in diameter with spike-like projections on their surface, giving them a crown-like appearance under the electron microscope, hence the name coronavirus.[1] Coronaviruses are divided into 4 classes designated as alpha, beta, gamma, and delta. Coronavirus disease 2019 (COVID-19), the novel disease, is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[2]; SARS-CoV-2 is a beta-coronavirus class, as are Severe Acute Respiratory Syndrome (SARS) coronavirus (SARS-CoV) and Middle East Respiratory Syndrome (MERS) coronavirus (MERS-CoV). The SARS-CoV-2 proteins, including the S protein, which is associated with entry into host cells and replication, are structurally similar to SARS-CoV. SARS-CoV-2 is known to infect the respiratory system and cause viral pneumonia, similar to SARS-CoV and MERS-CoV.[1] With similar clinical features as SARS and MERS, the most common symptoms of COVID-19 are fever, cough, chills/repeated shaking with chills, myalgia, headache, anosmia/ageusia, dyspnoea on effort, and sore throat. Some patients also develop lymphopenia and pneumonia with characteristic pulmonary ground glass opacity changes on chest computed tomography (CT) scan.[3] As with the prior outbreaks of SARS and MERS, numerous approaches are being undertaken in an attempt to treat and prevent the disease.

Current information indicates that SARS-CoV-2 is more transmissible than SARS-CoV.[4] The primary mode of infection is human-to-human transmission through respiratory droplets, aerosols, and close contact. COVID-19 has a probable asymptomatic incubation period between 1 and 14 days.[1]

Patients infected with SARS-CoV-2 present with a range of symptoms from asymptomatic infection to severe respiratory failure, septic shock, and multiple organ failure.[5] The most common symptoms are fever, cough, myalgia, and dyspnoea, though some patients present with headache, dizziness, nausea, and vomiting. Viral pneumonia occurs in severe disease and leads to severe acute respiratory failure. COVID-19 might trigger a “cytokine storm”, which may result in acute respiratory distress syndrome, respiratory failure, shock, multiple organ failure, and potentially death.[6] The mortality rates due to COVID-19 vary considerably from 0.03 to 192.39 per 100 000 population; the mortality rate is highest in San Marino (192.39 per 100 000 population) followed by Belgium (182.80 per 100 000 population); and the mortality rate in Spain is estimated to be 121.55 per 100 000 population.[7]

Plitidepsin

Plitidepsin (previously referred to as APLD or APL) is a cyclic depsipeptide originally isolated from a Mediterranean marine tunicate, *Aplidium albicans*. Plitidepsin is currently manufactured by total synthesis.

Plitidepsin has been approved in Australia for the treatment of multiple myeloma and is in development for treatment of COVID-19. The target for both its antitumour and antiviral activity is eukaryotic elongation factor 1A (eEF1A), which is one of the most abundant protein synthesis factors in eukaryotic cells. In its guanosine triphosphate (GTP)-bound form, eEF1A escorts aminoacyl-tRNA to ribosomes. Once associated with the ribosome, eEF1A hydrolyses GTP,

dissociates from the aminoacyl-tRNA, and leaves the ribosome to repeat the process[8], resulting in chain elongation.

As viruses depend on host cell proteins for their replication and propagation, it is not surprising that they have apparently evolved to utilise eEF1A in their life cycle.[9] One of the most abundantly produced proteins within eukaryotic cells infected with coronaviruses is the nucleocapsid (N) protein, a structural protein that forms complexes with genomic RNA, interacts with the viral membrane protein during virion assembly, and plays a critical role in enhancing the efficiency of virus transcription and assembly.[10] Coronavirus N protein was shown to interact directly with eEF1A and viral replication was inhibited by eEF1A knockdown or pharmacologic inhibition in host cells, suggesting the interaction between eEF1A and coronavirus N protein is essential.[11,12,13]

To determine whether the plitidepsin has an effect on SARS-CoV-2 virus replication, a set of *in vitro* experiments was performed. Briefly, human angiotensin converting enzyme 2 (hACE2)-stable transfected HEK293T cells were transfected with different plasmids expressing either EF1A wild type, EF2A (an elongation factor isoform not targeted by plitidepsin), or EF1A-A399V (which contains an amino acid mutation that induces resistance to plitidepsin structurally-related compounds).[14] Each of these hACE2-transfected HEK293T cell lines were infected with SARS-CoV-2 and the effect of plitidepsin treatment assessed. Results showed a 95-fold higher half-maximal inhibitory concentration (IC_{50}) for plitidepsin against infected cells transfected with EF1A-A399V compared with EF1A wild type or EF2A. Additionally, against EF1A-A339V CRISPR knock-in HEK293T-hACE2 cells infected with SARS-CoV-2, the plitidepsin IC_{50} was 141-fold higher than for the parental cell line. These results confirmed that plitidepsin inhibition of EF1A is the molecular basis for plitidepsin antiviral effects.

Preclinical pharmacology studies showed that plitidepsin is a potent inhibitor of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) growth *in vitro,* with half-maximal inhibitory concentration (IC_{50}) of 0.7 to 60 nM. Plitidepsin also demonstrated potent antiviral effects in two mouse models of SARS-CoV-2 infection, effecting over 2-log decrease in lung viral load and reduction in lung inflammation. Additionally, [redacted] and [redacted] studies showed that plitidepsin [redacted].

Plitidepsin pharmacokinetics (PK) were assessed in phase I studies evaluating different dosing schedules in patients with solid tumours and haematologic malignancies, and the regimen 1 h IV D1-5 q4wk was considered ideal for an infectious disease indication, maintaining sustained exposure and allowing daily adjustments based on the patient's response during the most critical days (1-3) to overcome disease. Results obtained through a validated population PK model of plitidepsin (PharmaMar data on file) simulating plitidepsin plasma profiles in typical subjects treated with the selected dose regimen of 1.5-h IV on D1-3 showed that flat doses of 1.5, 2.0, and 2.5 mg achieved mean plasma concentrations associated with lung exposure above the *in vitro* target concentration IC_{50} of 0.33 µg/L throughout the 3-day treatment period and remained above IC_{90} of 0.96 µg/L during most of the administration interval. These doses were used in a randomised, parallel, open-label, proof-of-concept study in 45 patients with confirmed SARS-CoV-2 infection. Plitidepsin treatment was well tolerated, with equivalent safety outcomes in all three dose cohorts. Two Grade 3 treatment-related adverse events were observed (hypersensitivity and diarrhoea). Treament-related adverse events affecting more than 5% of

patients were: nausea (42.2%), vomiting (15.6%), and diarrhoea (6.7%). Mean viral load reductions from baseline were 1.35, 2.35, 3.25, and 3.85 log_{10} at Days 4, 7, 15, and 31. Non-mechanical invasive ventilation was required in eight of 44 evaluable patients (16.0%); six patients required intensive care support (13.6%), and three patients (6.7%) died (COVID-19-related).

1.1. Study Rationale

The World Health Organization (WHO) declared COVID-19 a pandemic on 11 March 2020. COVID-19 is caused by a novel coronavirus, named SARS-CoV-2. An outbreak of SARS-CoV-2 started in Wuhan, China in December 2019.[15,16,17] The virus has subsequently spread throughout the world. In Europe, as of 12 December 2020, there were 21 413 786 confirmed cases of COVID-19 with 474 082 deaths.[18] On 22 October 2020, the United States (US) Food and Drug Administration (FDA) approved the antiviral drug Veklury (remdesivir) for use in adult and pediatric patients ≥12 years of age and weighing at least 40 kilograms for the treatment of COVID-19 requiring hospitalisation; first treatment for COVID-19 to receive FDA approval.[19] Since then, the US FDA has approved several EUA (the information is available on the FDA webpage).[20-25]

Remdesivir was authorised in the European Union in July 2020 as Veklury for the treatment of COVID-19 in adults and adolescents from 12 years of age with pneumonia who required supplemental oxygen.[26] The European Medicines Agency has endorsed the use of dexamethasone in adults and adolescents (from 12 years of age and weighing at least 40 kg) who require supplemental oxygen therapy.[27] Romania has permitted favipiravir use in special situations for adult patients with mild-to-moderate COVID-19 infection.[28]

Since July 2020, further treatments have been approved in the European Union. The information is available on the EMA webpage.[29]

Due to the high virulence and significant morbidity and mortality associated with SARS-CoV-2 infection, there is still an urgent unmet need for safe and effective drugs against SARS-CoV-2 that can reduce the viral burden in the upper respiratory tract and forestall complications of the infection. This study is justified based on the safety and efficacy findings from the APLICOV-PC proof-of-concept study, in which 45 patients hospitalised for COVID-19 infection were randomised to treatment with plitidepsin at doses of 1.5, 2.0, and 2.5 mg daily for 3 days.[30] Key findings are summarised briefly as:

- Safety assessments showed comparable results across all three dose groups. Importantly, the safety profile for COVID-19 patients treated with a cumulative dose of up to 7.5 mg plitidepsin (2.5 mg/day x 3) in the APLICOV-PC study was consistent with that established for cancer patients receiving their first cycle of treatment at the equivalent of 8.8 mg/day plitidepsin (5 mg/m^2 x 1.75 m^2 average body surface area) once every two weeks. The most commonly reported adverse events (AEs) included gastrointestinal disorders of constipation (18% *versus* 19% for COVID-19 and cancer patients, respectively), diarrhoea (18% *versus* 16%), nausea (42% *versus* 44%), and vomiting (18% *versus* 23%); constitutional symptoms of asthenia/fatigue (13% *versus* 62%) and pyrexia (44% *versus* 11%); nervous system disorders of dizziness (7% *versus* 5%) and

headache (13% *versus* 7%); and respiratory symptoms of cough (24% *versus* 14%) and dyspnoea (16% *versus* 13%).

- Quantitative polymerase chain reaction (qPCR) for SARS-CoV-2 viral load showed a mean (range) baseline viral load of 6.1 (1.5 to 10.6) log_{10} copies/mL, with a mean -2.35 log_{10} reduction at Day 7 and mean -3.25 log_{10} reduction at Day 15. These results support the conclusion that plitidepsin reduces viral replication.
- Efficacy assessments showed comparable results across all three dose groups, with 57% (25/44) of patients discharged from hospital by Day 8 and 82% (36/44) by Day 15. Post-hoc logistic regression analyses performed to assess covariates associated with hospital discharge by Day 15 found that only the covariate of baseline viral load was significantly correlated with hospital discharge by Day 15. The covariates of 2.5 mg/day plitidepsin dose and age showed a correlation that nearly reach significance. Consistent with results from the logistic regression analyses, differences in viral load at baseline were significantly correlated with efficacy outcomes, including use of oxygen therapy, use of non-invasive and invasive ventilation, and death, but viral load at baseline was not correlated with disease severity at baseline.
- Additionally, among patients with moderate severity COVID-19 infection (N=23), log-rank analysis of the probability of hospital discharge as a function of plitidepsin dose indicated a dose-related increased in discharge by Day 8, with 100% (8/8) of patients treated with 2.5 mg/day plitidepsin discharged by Day 8 compared with 57% (4/7) treated with 2.0 mg/day and 62% (5/8) treated with 1.5 mg/day.

Based on these findings and considering that all three doses appeared to be equally well tolerated, the low and high doses were selected for treatment of patients with moderate severity COVID-19 infection in the randomised, controlled phase 3 study to allow a rigorous assessment of the dose-response effect observed in the APLICOV-PC study.

1.2. Background

Plitidepsin is being investigated for management of patients with COVID-19. Preclinical and clinical pharmacology and toxicology studies indicated that plitidepsin has antiviral activity with tolerable safety profile.

In eukaryotic cells, FLIM-FRET experiments demonstrated that plitidepsin localises sufficiently close to eEF1A to suggest the formation of drug-protein complexes in the cytoplasm.[31] A separate set of experiments carried out with ^{14}C-plitidepsin and eEF1A purified from rabbit muscle showed that plitidepsin binds eEF1A with high affinity and a low rate of dissociation.

More detailed information about plitidepsin may be found in the Investigator's Brochure (IB).

Plitidepsin Activity on SARS-CoV-2 in Vitro

Several *in vitro* experiments aimed at determining the effect of plitidepsin on SARS-CoV-2 were carried out by independent research groups. Two independent laboratories, each using Vero E6 cells infected with SARS-CoV-2 and direct quantitation of SARS-CoV-2 nucleocapsid (N) protein, which is clearly involved in the mechanism of plitidepsin-induced antiviral activity,

showed that plitidepsin is a potent inhibitor of SARS-CoV-2 growth *in vitro*, with IC_{50} of 0.7 to 60 nM. In another study, human stem cell-derived pneumocyte-like cells were prophylactically exposed to 10 nm plitidepsin for 1 hour and then infected with SARS-CoV-2 (4×10^4 plaque forming units). After a 48-hour incubation period, both antiviral and cytotoxic plitidepsin effects were determined. Results showed that plitidepsin completely eliminated replication of SARS-CoV-2 with no observable cytotoxicity against the pneumocyte-like cells.

Plitidepsin Effects on SARS-CoV-2 in Vivo

Plitidepsin demonstrated potent antiviral effects *in vivo*, using previously described mouse models of adenovirus-mediated hACE2 and a transgenic mice expressing hACE2 driven by the cytokeratin-18 gene promoter (K18-hACE2), both infected with SARS-CoV-2.[32,33] In these experiments, results demonstrated that the treatment with plitidepsin induced a reduction of circa 2 log units in viral lung titers, as well as a reduction in lung inflammation.[32,33]

Plitidepsin Effects on Host Inflammatory Reaction

Similar to SARS-CoV, infection with SARS-CoV-2 also produces hypersecretion of several cytokines, with increasing plasma levels as the disease progresses, suggesting a possible relation between cytokine release and disease severity.[34,35]

Innate immunity is the first line of defence against invading pathogens. In the case of SARS-CoV-2, the entry of the virus into host epithelial cells is mediated by the interaction between the viral envelope spike (S) protein and the cell surface receptor ACE2. Viral RNAs, as pathogen-associated molecular patterns, are then detected by the host pattern recognition receptors, which include the family of toll-like receptors. Toll-like receptors then upregulate antiviral and proinflammatory mediators, such as interleukin (IL)-6, IL-8, and interferon (IFN)-γ, through activation of the transcription factor nuclear factor kappa B (NF-κB).[36,37] The importance of NF-κB towards proinflammatory gene expression, particularly in the lungs, has been highlighted by studies exploring SARS-CoV- infection in nonclinical species[38] as well as in patients.[39,40] In mice infected with SARS-CoV, the pharmacologic inhibition of NF-κB resulted in higher survival rates and reduced expression of tumour necrosis factor alpha (TNFα), chemokine ligand 2 (CCL2), and chemokine (C-X-C motif) ligand 2 (CXCL2) in lungs.[41]

Early *in vitro* studies showed that plitidepsin induces down-regulation of NF-κB pathway in tumour cells.[42] Subsequently, both *in vitro* and *ex vivo* studies were performed to assess the effects of plitidepsin on [redacted] cells.

In vitro studies were performed using [redacted] cells, a spontaneously immortalised [redacted]-like cell line derived from the [redacted] of a [redacted] case of [redacted] that is widely used for investigating [redacted] structure and function. Results showed that all the pathogen-associated molecular patterns-mimicking compounds induced the production of [redacted] in [redacted] cells and the addition of plitidepsin significantly reduced the secretion of the [redacted].

An *ex vivo* study assessed the effect of plitidepsin on expression of the [redacted], [redacted], and [redacted]α in the [redacted] of mice. Results showed that cluster of differentiation [redacted] cells

from placebo-treated mice were capable of producing [redacted], and [redacted] upon stimulation with [redacted]. However, [redacted] cells from plitidepsin-treated mice failed to show a marked increase in [redacted], and [redacted] compared with unstimulated controls. These results suggest that the *in vivo* exposure to plitidepsin prevented the increased production of [redacted] in the [redacted] cells isolated from [redacted]

Additional information about preclinical studies performed to evaluate pharmacokinetics (PK), safety pharmacology, and toxicology of plitidepsin may be found in the IB.

Safety in COVID-19 Patients

Study APLICOV-PC was a Phase 1, multicentre, open-label study in which 45 patients hospitalised for management of COVID-19 were randomised into 3 dose groups, comprising 1.5, 2.0, and 2.5 mg plitidepsin administered as a 1.5-hour IV infusion once a day for 3 consecutive days.[30] The primary objective of this study was to determine the safety and toxicological profile at each dose level, based on (1) frequency of Grade ≥3 treatment-emergent adverse events (TEAEs) at Days 3, 7, 15, and 31 according to National Cancer Institute (NCI)-Common Terminology Criteria for Adverse Events (CTCAE) version 5.0; (2) percentage of patients unable to complete treatment and reasons; (3) percentage of patients with TEAEs and serious adverse event (SAEs) at Days 3, 7, 15, and 31; and (4) change from baseline haematologic and non-haematologic parameters on Days 3, 7, 15, and 31. A secondary objective was to select a recommended dose for a pivotal study.

Findings for protocol-specified safety endpoints are summarised as follows:

- Grade ≥3 AEs: 31% (14/45) of patients experienced Grade ≥3 AEs (Table 1) and only 2 patients experienced a treatment-related Grade ≥3 AE: one case of anaphylactic reaction during the first plitidepsin infusion that resulted in treatment discontinuation and one case of diarrhoea that had no impact on plitidepsin treatment. Consistent with Grade ≥3 AEs being predominantly due to COVID-19 infection, the prevalence of Grade ≥3 AEs largely reflected the percentage of patients with mild disease in each cohort, with the highest prevalence in the 2.5-mg cohort (6.7% with mild disease, 40.0% Grade ≥3 AEs), a lower percentage in the 1.5-mg cohort (13.3% with mild disease, 33.3% Grade ≥3 AEs), and the lowest percentage in the 2.0-mg cohort (20.0% with mild disease, 20.0% Grade ≥3AEs). No events of special interest occurred, with the exception of one case of Grade ≥3 ALT elevation.
- Patients unable to complete treatment: Only one patient was unable to complete plitidepsin treatment, after experiencing an anaphylactic reaction within the first 5 minutes after starting infusion.
- Patients with SAEs: 22% (10/45) of patients experienced SAEs, including 6 dosed at 1.5 mg, 1 dosed at 2.0 mg, and 3 dosed at 2.5 mg (Table 2). With the exception of one case of anaphylactic reaction, all these events were related to COVID-19 infection.

- Patients with AEs: No dose-related trends were noted for any reported AEs. Importantly, the safety profile for COVID-19 patients treated with a cumulative dose of up to 7.5 mg plitidepsin (2.5 mg/day x 3) in the APLICOV-PC study was consistent with that established for cancer patients receiving their first cycle of treatment at the equivalent of 8.75 mg/day plitidepsin (5 mg/m^2 x 1.75 m^2 average body surface area) on a Days 1 and 15 q4w schedule. The most commonly reported AEs included gastrointestinal disorders of constipation (18% *versus* 19% for COVID-19 and cancer patients, respectively), diarrhoea (18% *versus* 16%), nausea (42% *versus* 44%), and vomiting (18% *versus* 23%); constitutional symptoms of asthenia/fatigue (13% *versus* 62%) and pyrexia (47% *versus* 11%); nervous system disorders of dizziness (7% *versus* 5%) and headache (13% *versus* 7%); and respiratory symptoms of cough (24% *versus* 14%) and dyspnoea (16% *versus* 13%).
- Changes in laboratory parameters: Although 62% of patients showed ≥1 grade change in haematology parameters and 84% of patients showed ≥1 grade change in chemistry parameters on study, few patients showed >1 grade shift. For haematology parameters, 6 patients had lymphopenia worsen by 2-3 grades and 2 had neutropenia worsen by 2-3 grades. For chemistry parameters, 5 patients had ALT increase by 2-3 grades, 2 had AST increase by 2-3 grades, 3 had gamma-glutamyl transferase (GGT) increase by 2-3 grades, and 2 had hypoglycaemia (glucose) increase by 2 grades.

Based on these findings, the Sponsor concluded that plitidepsin treatment was well tolerated and was unable to detect differences in safety between the 3 doses studied. Overall, the safety profile in the APLICOV-PC study for 1.5 to 2.5 mg Dx3 treatment was consistent with that established for cancer patients receiving their first cycle of treatment with the equivalent of 8.75 mg/day on a Days 1 and 15 Q4W schedule.

Table 1: Grade ≥ 3 Adverse Events, Regardless of Relationship, in APLICOV-PC Study

MedDRA SOC Preferred Term	Dose Cohort			
	1.5 mg N=15	**2.0 mg N=15**	**2.5 mg N=15**	Total N=45
	n (%)			
Patients with any Grade ≥ 3 AE	5 (33.3)	3 (20.0)	6 (40.0)	14 (31.1)
Blood and lymphatic system disorders				
Leukocytosis	---	---	1	1
Gastrointestinal system disorders				
Diarrhoea	---	---	1	1
General disorders & administration site conditions				
Condition aggravated	1	---	---	1
Immune system disorders				
Anaphylactic reaction	1	---	---	1
Infections and Infestations				
Bacteremia	---	---	1	1
COVID-19 pneumonia	---	---	1	1
Pneumonia	1	---	---	1
Superinfection bacterial	---	---	1	1
Urinary tract infection	---	---	1	1
Investigations				
ALT increased	---	1	---	1
C-reactive protein increased	1	---	---	1
GGT increased	1	2	---	3
Ferritin increased	1	---	---	1
Nervous system disorders				
Status epilepticus	---	---	1	1
Metabolism and nutrition disorders				
Hyperglycaemia	---	---	1	1
Respiratory, thoracic and mediastinal disorders				
Acute respiratory distress syndrome	2	1	1	4
Respiratory failure	---	---	1	1
Pneumomediastinum	---	---	1	1
Respiratory distress	---	---	1	1

---: no events reported
Note: each patient reported once per preferred term.

Table 2: Serious Adverse Events (SAEs) in APLICOV-PC Study

Subject ID	Sex	Age	Dose Cohort	SAE event	Relatedness	Study Day of Onset/Resolution
[redacted]	M	50	1.5 mg	Pyelonephritis	unrelated	Day 29/Day 37
[redacted]	M	40	1.5 mg	Acute respiratory distress syndrome	unrelated[A]	Day 6/Day 14
[redacted]	M	32	1.5 mg	Anaphylactic reaction	related	Day 1/Day 1
[redacted]	M	75	1.5 mg	Acute respiratory distress syndrome	unrelated	Day 6/Day 30
[redacted]	F	51	1.5 mg	Condition aggravated	unrelated	Day 6/Day 12
[redacted]	M	52	1.5 mg	COVID-19 pneumonia	unrelated[A]	Day 6/Day 19
[redacted]	M	71	2.0 mg	Acute respiratory distress syndrome	unrelated[A]	Day 5/Day 18
[redacted]	M	79	2.5 mg	COVID-19 pneumonia & pneumomediastinum	unrelated[A]	Day 09/Day 21 Day 20/Day 21
[redacted]	M	57	2.5 mg	Bacterial superinfection & respiratory failure	unrelated[A]	Day 18/Day 56
[redacted]	F	84	2.5 mg	Acute respiratory distress syndrome	unrelated[A]	Day 5/Day 14
				Status epilepticus	unrelated[B]	Day 51/Day 51

A: SAE attributed to COVID-19 infection; B: serious adverse event attributed to neuroleptic medication

Efficacy in COVID-19 Patients

A secondary objective of the study was to assess efficacy at each dose level based on (1) change in SARS-CoV-2 viral load from baseline (Day 1 prior to start of the study treatment) measured at Days 4, 7, 15, and 31; (2) time from baseline until undetectable SARS-CoV-2 viral load by reverse transcription polymerase chain reaction (RT-PCR); (3) mortality at Days 7, 15, and 31; (4) percentage of patients requiring invasive mechanical ventilation and/or ICU admission at Days 7, 15, and 31; (5) percentage of patients requiring non-invasive mechanical ventilation at Days 7, 15, and 31; and (6) percentage of patients requiring oxygen therapy at Days 7, 15, and 31. Results of these assessments, summarised in Table 3, showed comparable results across the three dose groups. As progressive deterioration of respiratory function and development of cytokine release syndrome typically occur at a mean of 10 days from onset of COVID-19 symptoms[44], the endpoint of hospital discharge by Day 15 is considered to reflect successful amelioration of life-threatening complications.

Considering that dose groups were reasonably balance for baseline characteristics, including age, gender, time from symptom onset to randomisation, comorbidities associated with increase susceptibility to complications from COVID-19 infection, disease severity at baseline, vital signs and laboratory assessments at baseline, and viral load at baseline, post-hoc analyses were performed to identify factors associated with hospital discharge by Day 15. Logistic regression analyses performed to assess a wide range of covariates for correlation with hospital discharge by Day 15 found that only the covariate of viral load at baseline was significantly correlated with

discharge rate. Additionally, covariates of 2.5-mg plitidepsin dose and age showed correlations that nearly reached significance. Consistent with results from the logistic regression analyses, differences in viral load at baseline were significantly correlated with efficacy outcomes, including use of oxygen therapy, use of invasive and non-invasive mechanical ventilation, and death, but viral load at baseline was not correlated with disease severity at baseline.

Among patients with moderate severity COVID-19 infection (N=23), log-rank analysis of the probability of hospital discharge as a function of plitidepsin dose indicated a dose-related increased in discharge by Day 8, with 100% (8/8) of patients treated with 2.5 mg/day plitidepsin discharged by Day 8 compared with 57% (4/7) treated with 2.0 mg/day and 62% (5/8) treated with 1.5 mg/day.

Table 3: Summary of Protocol-specified Efficacy Endpoints in Study APLICOV-PC

Endpoint	Dose Cohort			
	1.5 mg N=14[A]	2.0 mg N=15	2.5 mg N=15	Total N=44
Patients discharged from hospital	n (%)			
Day 1 to Day 7	3 (21.4)	2 (13.3)	5 (33.3)	10 (22.7)
Day 1 to Day 8	6 (42.9)	9 (60.0)	10 (66.7)	25 (56.8)
Day 9 to Day 15	5 (35.7)	5 (33.3)	1 (6.7)	11 (25.0)
Day 1 to Day 15	11 (78.6)	14 (93.3)	11 (73.3)	36 (81.8)
Day 1 to Day 31	13 (92.9)	14 (93.3)	13 (86.7)	40 (90.9)
Mortality from Day 1 to				
Day 7	---	---	---	---
Day 15	---	---	---	---
Day 31[B]	1 (7.1)	---	1 (6.7)	2 (4.5)
Patients requiring invasive mechanical ventilation and/or ICU admission				
Day 1 to Day 7	2 (14.3)	1 (6.7)	2 (13.3)	5 (11.4)
Day 8 to Day 15	1 (7.1)	1 (6.7)	1 (6.7)	3 (6.8)
Day 16 to Day 31	1 (7.1)	1 (6.7)	1 (6.7)	3 (6.8)
Day 1 to Day 31	2 (14.3)	1 (6.7)	3 (20.0)	6 (13.6)
Patients requiring non-invasive mechanical ventilation				
Day 1 to Day 7	4 (28.6)	0	1 (6.7)	5 (11.4)
Day 8 to Day 15	3 (21.4)	0	2 (13.3)	5 (11.4)
Day 16 to Day 31	1 (7.1)	1 (6.7)	1 (6.7)	3 (6.8)
Day 1 to Day 31	5 (35.7)	1 (6.7)	2 (13.3)	8 (18.2)
Patients requiring oxygen therapy at				
Day 7	12 (85.7)	12 (80.0)	11 (73.3)	35 (79.5)
Day 15	4 (28.6)	1 (6.7)	4 (26.7)	9 (20.5)
Day 31	0	2 (13.3)	1 (6.7)	3 (6.8)
Days 1 to 31	12 (85.7)	12 (80.0)	11 (73.3)	35 (79.5)
Mean change in viral load from baseline to[C]	log_{10} copies/mL			
Day 4	-1.23	-1.49	-1.32	-1.35
Day 7	-2.55	-2.26	-2.25	-2.35
Day 15	-4.22	-2.70	-2.92	-3.25

Day 31	-4.70	-3.53	-3.49	-3.85
		days		
Mean time from baseline until undetectable viral load[C]	11	14	14	13

Abbreviations: --- = no events reported; ICU = intensive care unit

A: Patient who experienced an anaphylactic reaction during the first plitidepsin infusion had treatment discontinued and was not considered evaluable for efficacy

B: One additional patient treated at 2.5 mg/day died on Day 57, because of COVID-19 complications.

C: Results based on 42 patients at Day 4 (13 at 1.5 mg, 14 at 2.0 mg, 15 at 2.5 mg), 40 patients at Day 7 (13 at 1.5 mg, 14 at 2.0 mg, 13 at 2.5 mg), 38 patients at Day 15 (12 at 1.5 mg, 13 at 2.0 mg, 13 at 2.5 mg), and 39 patients at Day 31 (11 at 1.5 mg, 14 at 2.0 mg, 14 at 2.5 mg)

1.3. Benefit-risk Assessment

In the APLICOV-PC study, most patients (87%) had moderate-to-severe disease and 82% of patients were discharged by Day 15. As progressive deterioration of respiratory function and development of cytokine-release syndrome typically occur at a mean of 10 days from onset of symptoms,[44] the endpoint of hospital discharge rate at Day 15 is considered to reflect successful amelioration of life-threatening complications. Post-hoc analyses showed that only baseline viral load was significantly correlated with hospital discharge by Day 15.

In the APLICOV-PC study, median baseline viral load was 6.1 (1.5 to 10.6) $\log_{10}$ copies/mL a mean -3.25 $\log_{10}$ reduction in viral load was observed by Day 15. These results support the conclusion that plitidepsin reduces viral replication.

However, among patients with moderate disease at baseline, log-rank analysis showed a dose-response effect, with 100% (8/8) treated with 2.5 mg/day plitidepsin discharged by Day 8 compared with 57% (4/7) treated with 2.0 mg/day and 62% (5/8) treated with 1.5 mg/day.

Considering the low rate of drug-related Grade ≥3 AEs and the high discharge rate at Day 15, along with an average 3.25-$\log_{10}$ reduction in baseline viral load by Day 15, evidence to date indicates a positive benefit-risk for plitidepsin for treatment of patients hospitalised for COVID-19 infection and justifies conducting a randomised, controlled trial to establish tolerance and efficacy.

As plitidepsin is a substrate of cytochrome P450 3A4 (CYP3A4), strong inhibitors and inducers of CYP3A4 should not be co-administered with plitidepsin unless there is no therapeutic alternative. Moderate inhibitors and inducers of CYP3A4 should be used with caution in combination with plitidepsin (Appendix 4 – Inhibitors and Inducers of CYP3A4).

Additional information about risks based on preclinical findings, clinical findings, and AEs of special interest are provided in the IB.

2. OBJECTIVES AND ENDPOINTS

2.1. Objectives

Primary objective

- To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control assessing the need of supplementary oxygen.

Key secondary objective

- To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control assessing the time to sustained hospital discharge.

Secondary Objectives

- To compare efficacy of plitidepsin 1.5 and 2.5 mg *versus* control:
  - Assessing clinical status by the 11-category WHO Clinical Progression Scale[45] (See Appendix 13 - World Health Organization (WHO) Clinical Progression Scale).
  - In terms of the need of advanced oxygen support.
  - In terms of need for intensive care support.

- To compare safety/tolerability of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of:
  - adverse events, adverse reactions and mortality.
  - abnormal laboratory parameters.
  - variations of vital signs.
  - ECG variations.

Other secondary objectives

- To compare efficacy of plitidepsin 1.5 mg or 2.5 mg *versus* the control in terms of:
  - hospital readmission related to COVID-19.
  - clinical evolution.
  - the need of supplementary oxygen.
  - the need of intensification of respiratory or intensive care support.
  - the need of intensification of pharmacological therapies.
  - superinfections.
  - mortality.
  - the evolution of viral load.
  - the evolution of inflammatory markers.
  - the immune response against SARS-CoV-2.

- To compare efficacy in the primary endpoint and describe safety/tolerability of pooled plitidepsin arms *versus* control.
- To compare efficacy in the primary endpoint and describe safety/tolerability between plitidepsin arms (1.5 *versus* 2.5 mg) in case both are significantly superior to the control.

- To explore the influence of risk factors or scores for clinical deterioration that were not individually included.
- To compare the results obtained before and after the change of the primary and secondary endpoint (protocol v.6 *versus* v.7).

2.2. Endpoints

Primary efficacy endpoint

- Time to sustained withdrawal of supplementary oxygen (11-category WHO Clinical Progression Scale ≤4) with no subsequent reutilisation during remaining study period (See Appendix 13 - World Health Organization (WHO) Clinical Progression Scale).

Key secondary efficacy endpoint

- Time to sustained (i.e., with no subsequent readmission to Day 31) hospital discharge (since randomisation).

Secondary endpoints

Efficacy:

- Clinical status, as assessed by the 11 category WHO Clinical Progression Scale, at Day 8 (±1) (See Appendix 13 - World Health Organization (WHO) Clinical Progression Scale).
- Total duration of advanced oxygen support (high-flow nasal oxygen, extracorporeal membrane oxygenation -ECMO-, non-invasive ventilation or mechanical ventilation).
- Percentage of patients in each study group requiring admission to ICU on Days 4, 8, 15, and 31.

Safety/tolerability:

- Frequency of TEAEs;
- Frequency of TEAEs of ≥ 3 according to NCI-CTCAE v5.0;
- Frequency of treatment-emergent adverse events of special interest (AESIs);
- Frequency of serious adverse events (SAEs);
- Frequency of drug-related serious adverse events (i.e., SARs);
- Frequency of adverse events leading to treatment discontinuation;
- Frequency of deaths;
- Change respect to baseline in individual study-defined laboratory parameters (See Section 7.2.4.);
- Change respect to baseline in individual vital signs (See Section 7.2.5.);
- Change respect to baseline in individual study-defined ECG parameters (See Section 7.2.5.).

Other secondary endpoints

- Percentage of patients in each study group requiring hospital readmission due to COVID-19-related signs or symptoms through Day 31.

- Percentage of patients in each study group and in each of the categories of the 11-category WHO Clinical Progression Scale on Days 4, 8, 15 and 31 (See Appendix 13 - World Health Organization (WHO) Clinical Progression Scale).
- Percentage of patients in each study group requiring oxygen therapy on Days 4, 8, 15 and 31.
- Time to intensification of respiratory support (WHO >6 [intubation]) (See Appendix 13 - World Health Organization (WHO) Clinical Progression Scale).
- Total duration of intensive care unit (UCI) stay for each study group.
- Percentage of patients in each study group requiring high-flow oxygen on Days 4, 8, 15, and 31.
- Percentage of patients in each study group requiring non-invasive mechanical ventilation on Days 4, 8, 15, and 31.
- Percentage of patients requiring either extracorporeal membrane oxygenation ECMO or invasive mechanical ventilation by Days 4, 8, 15, and 31.
- Time to initation with immune-modulating drugs.
- Time to initiation with antiviral drugs.
- Percentage of patients receiving subsequent immune-modulating drugs on Days 4, 8, 15 and 31.
- Percentage of patients receiving subsequent antiviral drugs on Days 4, 8, 15 and 31.
- Percentage of patients in each study group with nosocomial infection by Days 4, 8, 15 and 31.
- Mortality in each study group on Days 4, 8, 15 and 31.
- Change in SARS-CoV-2 viral load in each study group from Day 1 before administration of the study drug until Day 8.
- Percentage of patients in each study group with undetectable SARS-CoV-2 viral load on Day 8.
- Change in inflammatory biomarkers (C-reactive protein [CRP], ferritin, interleukin [IL]-6, IL-1β, IL-10 and tumour necrosis factor alpha [TNFα]) in each study group from baseline until Days 2, 3, 4, 8, and 31.
- Change respect to baseline in individual serological assessments.
- Obesity, hypertension, age and individual co-morbidities included in the Charlson Index (Appendix 11- Age-adjusted Charlson Index), ISARIC-4C score (Appendix 14 - ISARIC 4C Mortality & 4C Deterioration Scores), or vaccination status.
- Time to sustained withdrawal of supplementary oxygen (11-category WHO Clinical Progression Scale ≤4) with no subsequent reutilisation during remaining study period, before (protocol v.6) and after the amendment (protocol v.7).
- Time to sustained (i.e., with no subsequent readmission to Day 31) hospital discharge (since randomisation), before (protocol v.6) and after the amendment (protocol v.7).

Additionally, a dedicated QTc substudy will be performed in a subset of approximately 50 patients with ECGs collected over Days 1 to 3 using Holter monitors to assess treatment impact on QTc prolongation. See Appendix 8 for objectives and respective endpoints planned for the QTc substudy. The substudy patients will have blood sampling for pharmacokinetic assessments over Days 1 to 4.

3. INVESTIGATION PLAN

3.1. Overall Study Design and Plan Description

An overview of study design is shown in Figure 1. This is a multicentre, open-label, controlled Phase 3 study in which adults requiring hospital admission and O_2 supplementation for management of moderate COVID-19 infection will be randomised in 1:1:1 to:

- Plitidepsin 1.5 mg arm: Patients will receive plitidepsin 1.5 mg/day intravenous (IV) in addition to dexamethasone on days 1 to 3 (See Section 5. Study treatments).
- Plitidepsin 2.5 mg arm: Patients will receive plitidepsin 2.5 mg/day IV in addition to dexamethasone on days 1 to 3 (See Section 5. Study treatments).
- Control arm: Patients will receive dexamethasone IV on Days 1 to 3. Additionally, in accordance with local treatment guidelines, patients in this group may receive a regulatory-approved antiviral treatment (See Section 5. Study treatments).

Randomisation will be stratified for 3 factors:

a) Geographical Region (Europe *versus* Rest of the World);
b) Charlson Comorbidity Index (0-1 *versus* >1) (Appendix 11 – Age-adjusted Charlson Index); and
c) Barthel Index (≥90 *versus* <90) (Appendix 7 - Barthel Index for Functional Assessment).

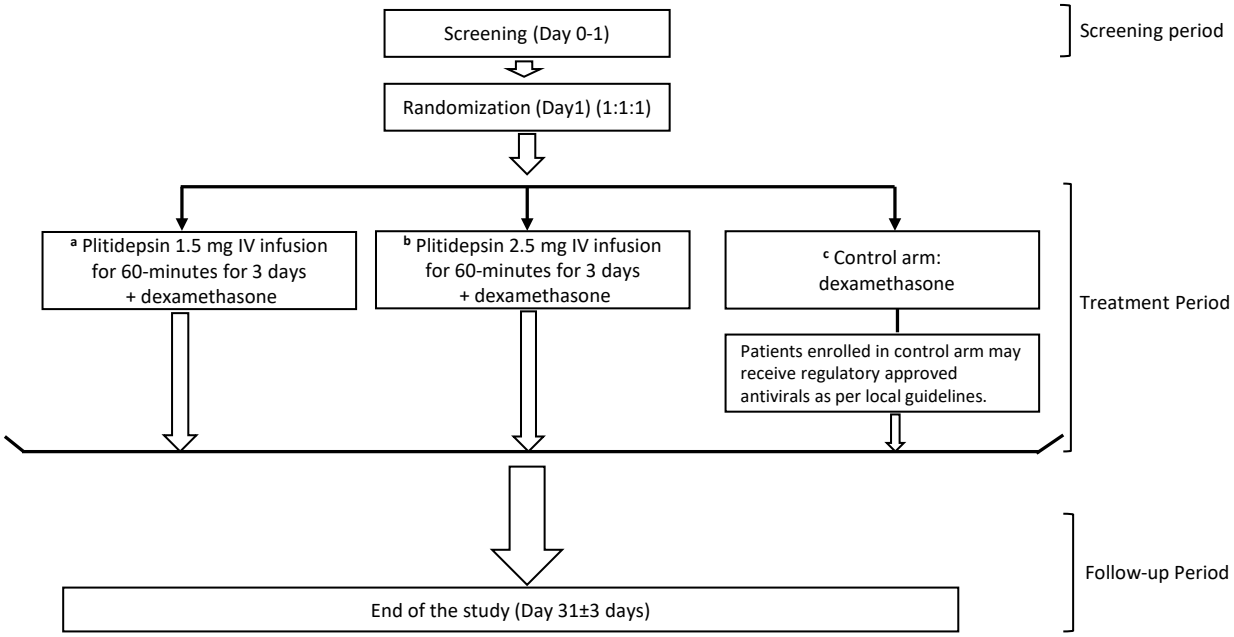
From treatment initiation on Day 1, patients will be followed in the hospital for at least 4 days and then through Day 31 (±3 days) or resolution/stabilisation of treatment-related adverse events, treatment-emergent adverse events of special interest, and SAEs. Patients discharged from the hospital prior to Day 8 will return to an out-patient clinic for assessments on Days 8 (±1 day) and 31 (±3 days). On Day 15 (±1 day), patients will be followed up in remote or on-site visit (Appendix 5 – Schedule of Assessments).

An Independent Data Monitoring Committee (IDMC) will oversee study conduct (safety and primary endpoint), including analysis of summary safety data as per the trial requirements.

The study is expected to randomise approximately 609 patients (203 in each arm) over a period of 20 months.

Patients will be considered to be on study from the signature of the informed consent form (ICF) until the end of the follow-up period, that is, completion of the protocol-specified follow-up visit on Day 31 (±3 days) or until resolution/stabilisation of treatment-related AEs, treatment-emergent adverse events of special interest, and SAEs that occurred through Day 31, whichever is longer.

Figure 1. Schema of the Study



Abbreviations: IV = intravenous
Notes: The study allows up to a total cumulative dose of 60 mg of dexamethasone base (calculation of the total dose will also include corticosteroids administered within 72 hours before the start of the study treatment and dexamethasone administered as premedication).
[a] Plitidepsin 1.5 mg arm: Patients will receive plitidepsin 1.5 mg/day intravenous (IV) in addition to dexamethasone on days 1 to 3.
[b] Plitidepsin 2.5 mg arm: Patients will receive plitidepsin 2.5 mg/day IV in addition to dexamethasone on days 1 to 3.
[c] Control arm: Patients will receive dexamethasone IV on Days 1 to 3. Additionally, in accordance with local treatment guidelines, patients in this group may receive a regulatory-approved antiviral treatment. Antiviral treatment to be used according to the approved product information in each country, different dosages could be used.

3.1.1. Independent Data Monitoring Committee

An IDMC will be established to provide study oversight considering that this is a multicentre, randomised study being performed in a population at high risk for morbidity and mortality. The IDMC will be established and operated in compliance with the FDA Guidance for Industry "*Establishment and Operation of Clinical Trial Data Monitoring Committees"*.

The IDMC will be composed of individuals external to the study sponsor, trial managers (including sponsor's and contract research organisation's [CRO] medical monitors), and study investigators, and will be comprised of at least 1 clinician specialised in the treatment of COVID-19 patients, 1 clinician specialised in assessment of clinical study safety issues, and 1 biostatistician specialised in analysis of clinical trials. One IDMC member will serve as chair and will prepare minutes of each IDMC meeting, which will be provided to the study sponsor, sponsor's medical monitor, and CRO project manager only at the end of the study.

An initial open meeting/teleconference between the study sponsor, sponsor's medical monitor, and IDMC will be held before enrolling the first patient on study to discuss the protocol and analytic plan, ICF, and plans for IDMC monitoring of study safety and effectiveness data.

The IDMC will have responsibility for:

- Review of all SAEs.
- Review of safety trends, such as an accumulating number of deaths in the study (i.e., from administration of the first dose of study drug on Day 1 through Day 31), to determine if there is a difference between plitidepsin and control groups and potential impact on study conduct.
- A futility analysis for efficacy/safety will be performed when 33% of events have been reached. The rho family of beta-spending functions (with ρ=1.18) will be used for the calculation of the stopping boundaries for futility in order to control type II error. The stopping boundaries for the futility analysis will be calculated with the actual number of events available in the ITT population at that moment; if the sample events at the futility analysis is 116 in a pair comparison, the beta-spending corrected p-value to reject the alternative hypothesis should be higher or equal than 0.4199 and this p-value is associated with a HR≤1.0382.

If the recommendation of the IDMC is to discontinue one of the plitidepsin dosing arms at this futility analysis following the stopping rules, the study will continue from that point on a 1:1 randomisation fashion until the completion of the remaining treatment arms (203 patients per arm and 353 events of sustained withdrawal of supplementary oxygen in total necessary to perform the final analysis). The patients still ongoing in the dropped arm would continue to be followed up as per protocol and a Bonferroni-adjusted type I error rate of 1.25% will be kept for the comparison of the primary endpoint between the remaining plitidepsin arm and the control arm.

In addition, at the time when 30 patients have been treated per group with a 30-day follow-up, the IDMC Support Team will provide the IDMC Chairperson with unblinded information on the number of deaths and SAEs. Upon review of this data, if either the difference in the percentage of patients with deaths or SAEs Grade ≥3 is at least 20% higher in any IMP group in comparison with the Control Group, IDMC Chairperson will contact the other members of the IDMC and the clinical trial would be halted for a specific safety analysis by the IDMC if she/he deems it necessary.

The IDMC can ask for a temporary halt of the clinical trial at any time to better analyse any potential benefit/risk concern.

A formal IDMC charter will be finalised before study initiation.

3.2. Discussion of Study Design, Including the Choice of Control Groups

This is a randomised, open-label, Phase 3 study in adult patients requiring hospitalisation and O_2 supplementation for management of moderate COVID-19 infection. The rationale for this study is outlined in Section 1.1. The study is designed to obtain safety and efficacy of both low dose (1.5 mg IV) and high dose (2.5 mg IV) plitidepsin. Because there were no clear differences in safety/tolerability and efficacy results for the 3 plitidepsin dose levels (1.5, 2.0, and 2.5 mg) evaluated in the APLICOV-PC study, this study design will help to assess the efficacy and safety

of both low dose (1.5 mg) and high dose (2.5 mg), and it will be compared with control arm for COVID-19. The target population of this study is adult patients requiring hospitalisation and O_2 supplementation for management of moderate COVID-19 infection.

All patients will receive dexamethasone phosphate 8 mg/day IV (equivalent to 6.6 mg dexamethasone base) on Days 1 to 3 (administered as a premedication in plitidepsin arms), followed by dexamethasone phosphate 7.2 mg/day (equivalent to 6 mg/day dexamethasone base) PO/IV from Day 4 and up to a total cumulative dose of 60 mg of dexamethasone base (as per physician judgement according to patient clinical condition and evolution).

Additionally, in accordance with local treatment guidelines, patients randomised to the control arm may receive a regulatory-approved antiviral treatment, such as remdesivir (200 mg IV on Day 1 followed by 100 mg/day IV on Days 2 to 5) or favipiravir (1600 mg BID PO on Day 1, followed by 600 mg BID PO daily for 2 to 5 days).

Best supportive care, consistent with National Institute of Health COVID-19 Treatment Guidelines (www.covid19treatmentguidelines.nig.gov) or local country guidelines will be provided to all study participants.

3.3. End of Study Definition

The study is considered completed after the last patient has completed all protocol-specified evaluations, including the protocol-specified Day 31 (±3 days) follow-up or until resolution/stabilisation of treatment-related adverse events, treatment-emergent adverse events of special interest, and SAEs that occurred through Day 31, whichever is longer.

3.4. Selection of Doses in the Study

Selection of doses for the Phase 1 APLICOV-PC study in patients hospitalised for COVID-19 infection was based on the rationale described below.

The antiviral activity of plitidepsin was tested *in vitro* against a variety of cells infected with SARS-CoV-2 virus. One of the first experiments using Vero E6 cells showed an IC_{50} of 3.265 (95% confidence interval [CI]: 2.973, 3.585) nM and a Hill slope (H) of 2.082. Using the below formula, 90% maximal inhibitory concentration (IC_{90}) was estimated as 9.38 (95% CI: 7.65, 11.50) nm in Vero cells.

$$\mathrm{ICF}_{in\ vitro} = \left(\frac{\mathrm{F}}{100 - \mathrm{F}}\right)^{1/\mathrm{H}} \times \mathrm{IC50}_{in\ vitro}$$

Plasma protein binding of plitidepsin was estimated as 98% and 96% in human and rat plasma, respectively.

An *in vivo* tissue distribution study in rats administered a single IV bolus dose of 0.2 mg/kg ^{14}C-plitidepsin showed significantly increased distribution of radioactivity into lungs, with a lung-to-blood ratio (LBR) of approximately 8 and a lung-to-plasma partition coefficient ratio of approximately 543.[46] The partition coefficient ratio enables quantification of the total drug concentration in tissue.

By assuming similar lung distribution in humans as observed in rodents and given a similar fraction of unbound drug in plasma and tissue in humans and rats, unbound exposures in human lung can be estimated using the equation

$$\mathrm{ICF}_{\mathrm{total,plasma}} = \frac{\mathrm{ICF}_{\mathrm{total},\,in\ vitro}}{\mathrm{f}_{\mathrm{u,\,human}} \cdot \mathrm{LBR}_{\mathrm{rat}}}$$

where $ICF_{total,plasma}$ is the total target plasma concentration (µg/L), $ICF_{total,in\ vitro}$ is the concentration (µg/L) used in the *in vitro* experiment, $f_{u,human}$ is the unbound fraction in human plasma, and LBR_{rat} is the lung-blood ratio in the distribution study in rats, the total plasma concentration (µg/L) of plitidepsin associated with lung exposure above the *in vitro* target concentration IC_{50} and IC_{90} were estimated at 0.33 (95% CI: 0.30, 0.37) µg/L and 0.96 (95% CI: 0.78, 1.18) µg/L, respectively, in Vero cells.

Plitidepsin PK was assessed in Phase 1 studies evaluating different dosing schedules in patients with solid tumours and haematologic malignancies. The dosing schedules examined, dose intensity, maximum tolerated dose, and recommended dose for further studies are summarised in Table 4. A validated population PK model showed no link between body surface area and plasma clearance, so flat dosing is considered preferable.

Of all the dosing regimens studied, the regimen of 1-hour IV on Days 1 to 5 Q4W was considered ideal for an infectious disease indication, maintaining sustained exposure and allowing daily adjustments based on the patient's response during the most critical days (1 to 3) to overcome disease.

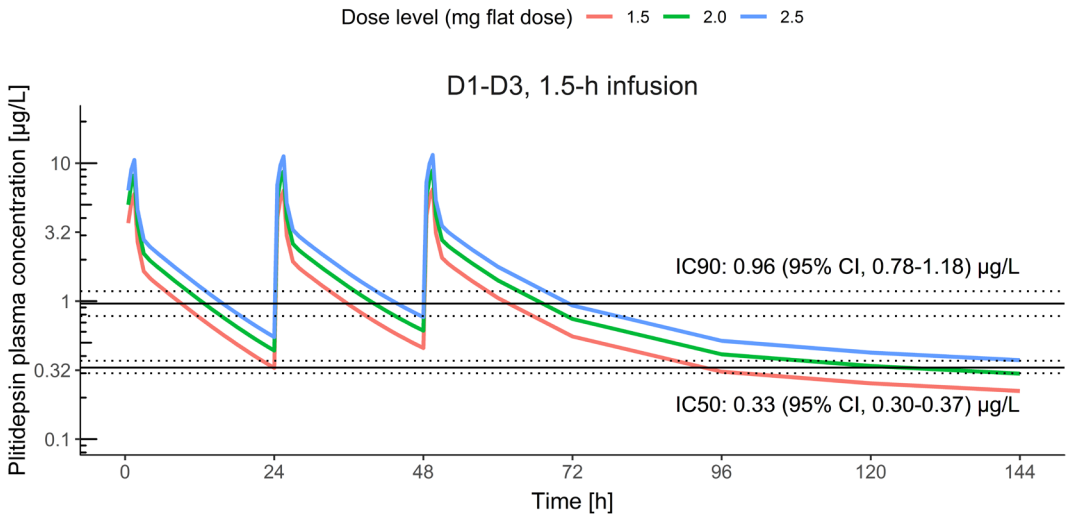
Table 4: Phase 1 Studies of Plitidepsin as a Single-agent in Patients with Solid Tumours and Haematologic Malignancies

Dosing regimen	24-h IV D1, 8, 15 Q4W	3-h IV D1, 15 Q4W	1-h IV D1, 8, 15 Q4W	24-h IV D1, 15 Q4W		1-h IV D1 to 5 Q3W
				Plitidepsin	Plitidepsin+L-carnitine	
Number of patients	35	27	48	67	20	37
MTD (mg/m^2)	4.5	6	3.6	6	8	1.35
RD (mg/m^2)	3.75	5	3.2	5	7	1.2
Dose intensity at RD (mg/m^2/week)	2.8	2.5	2.4	2.5	3.5	2.0

Abbreviations: D = day; h = hour; IV = intravenous; MTD = maximum tolerated dose; Q3W = every 3 weeks; Q4W = every 4 weeks; RD = recommended dose for further studies

A validated population PK model of plitidepsin (Pharma Mar data on file) was used to simulate plitidepsin plasma profiles in typical subjects treated with the selected dose regimens of 1.5-hour IV on Days 1 to 3 to observe whether they would achieve estimated target plasma concentrations with antiviral activity set at 0.33 µg/L (IC_{50}) and 0.96 µg/L (IC_{90}). Results, presented in Figure 2, showed that flat doses of 1.5, 2.0, and 2.5 mg were associated with plasma concentrations above IC_{50} throughout the 3-day treatment period and remained above IC_{90} during most of the administration interval. Accumulation after 3 repeated daily administrations is minimal. Moreover, the cumulative doses associated with the proposed administration schedule (4.5, 6.0, and 7.5 mg) are below the cumulative doses (8.4 to 12.0 mg) recommended on the basis of safety assessments for patients with advanced solid tumours receiving plitidepsin on a D x 5 Q3W schedule in a small Phase 1 study.[47]

Figure 2. Plitidepsin Plasma Concentration *versus* Time Profiles



Abbreviations: CI = confidence interval; D = day; h = hour; IV = intravenous; IC_{50} = half-maximal inhibitory concentration; IC_{90} = 90% maximal inhibitory concentration
Note: Plitidepsin plasma concentration *versus* time profiles predicted for a dosing schedule of 1.5-h IV on Days 1 to 3 at doses of 1.5, 2.0, and 2.5 mg using a validated population PK model. The horizontal black lines represent the total plasma concentrations associated with concentrations in lungs equivalent to IC_{50} and IC_{90} and *in vitro*.

4. SELECTION OF STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.

4.1. Inclusion Criteria

Patients must fulfil all of the following requirements to enter the study:

1. Signed informed consent obtained prior to initiation of any study-specific procedures and study treatment.
2. Documented diagnosis of SARS-CoV-2 infection, determined by either qualitative polymerase chain reaction (PCR), antigen test by local laboratory or any other validated method approved by the local health authority, from appropriate biological samples collected no more than 72 hours prior to study treatment on Day 1.
3. Patient meets category 5 on the 11-point WHO Clinical Progression Scale (Appendix 13 - World Health Organization (WHO) Clinical Progression Scale): requires hospitalisation and oxygen by mask or nasal prongs/cannula.
4. A maximum of 14 days from onset of COVID-19 symptoms to initiation of study treatment on Day 1.
5. Male or female aged ≥18 years.
6. Adequate bone marrow, liver, kidney, and metabolic function, defined by the following tests performed at local laboratory:
   - Absolute neutrophil count ≥500/mm^3 (0.5 x 10^9/L).
   - Platelet count ≥75,000/mm^3 (75 x 10^9/L).
   - ALT and AST ≤3 x upper limit of normal (ULN).
   - Serum bilirubin ≤1 x ULN (or direct bilirubin <1 x ULN when total bilirubin is above ULN).
   - Calculated creatinine clearance ≥30 mL/min (Cockcroft-Gault equation).
   - CPK ≤2.5 x ULN except if the patient has had recent (i.e, in the last week) shivering episodes or trauma. In that case, the level of CPK should be ≤5 x ULN).
7. Agree not to participate in another interventional clinical trial through Day 31.
8. Females of reproductive capacity must have a negative serum or urine pregnancy test by local laboratory at study enrolment and must be non-lactating.
9. Females and males with partners of child-bearing potential must use effective contraception while on study treatment and for 6 months after last dose of plitidepsin. Patients in the control arm must use effective contraception at the time indicated in the approved product information (summary of product characteristics [SmPC] or leaflet). If no information is available in the approved product information, patients in the control arm must use effective contraception for at least one week after the study completion or the time indicated based on the investigator's discretion.

4.2. Exclusion Criteria

The presence of any of the following criteria excludes a patient from participating in the study:

1. Subjects with a pre-baseline (i.e., in the month preceding the current COVID-19 infection) impairment in general health condition for whatever reason except COVID-19, with a severe dependency for daily living activities (Barthel index <60/100, Appendix 7 - Barthel Index for Functional Assessment) or chronic oxygen therapy.
2. Having received treatment for COVID-19 in another clinical trial in the prior 4 weeks, except documented allocation in a placebo arm.
3. Evidence of respiratory failure at the time of randomisation, based on resource utilisation requiring at least one of the following: endotracheal intubation and mechanical ventilation, oxygen delivered by high-flow nasal cannula, non-invasive positive pressure ventilation, ECMO, or clinical diagnosis of respiratory failure (i.e., clinical need for one of the aforementioned therapies, which could not be administered in a resource-limited setting).
4. Patients with severe COVID-19, meeting score >5 on the 11-point WHO Clinical Progression Scale (Appendix 13 - World Health Organization (WHO) Clinical Progression Scale) or presenting, after an initial stabilisation prior to randomisation, any of clinical signs indicative of severe systemic illness, such as respiratory rate ≥30 per minute, heart rate ≥125 per minute, or PaO_2/FiO_2 <300. In case a direct measure of PaO2 has not been obtained, it should be imputed according to referenced formula (Appendix 9 - Imputation of PaO_2 from SpO_2). For sites located over 1,000 m above sea level, PaO2/FiO2 ratio will be adjusted (Appendix 10 - Adjustment of PaO_2 from a Site at High Altitude; see also Appendix 12 - Imputation of FiO_2 from oxygen flow values. High-flow oxygen therapy).
5. Patients receiving, at randomisation, treatment with antiviral therapy against SARS-CoV-2 or requiring anti-inflammatory/immunomodulating drugs beyond glucocorticoids with the exception listed below:
   - Prior administration of dexamethasone or equivalent glucocorticoid might be acceptable if:
     - The total daily dose is no higher than 10 mg of dexamethasone phosphate (equivalent to dexamethasone base 8.25 mg/day) or equivalent glucocorticoids (Appendix 15 – Glucocorticoid equivalent anti-inflammatory doses).
     - The duration of the treatment does not exceed 72 hours prior to study treatment Day 1.
   - Prior administration of an antiviral might be acceptable in the following circumstances:
     - For small molecules (e.g., remdesivir, molnupiravir, nirmaltrevir/ritonavir), they must have been given for an earlier stage of the disease, outside a clinical trial, and there should be a documentation of objective clinical deterioration plus evidence of persisting positivity for SARS-CoV-2 in appropriate biological samples. Last dose of previous antiviral drugs should have been administered at least 24 h before randomisation.

- For antiviral monoclonal antibodies, they must have been given for an earlier stage of the disease (including pre-exposure prophylaxis), outside a clinical trial, and there should be a documentation of objective clinical deterioration plus evidence of persisting positivity for SARS-CoV-2 in appropriate biological samples. Last dose of antiviral monoclonal antibodies should have been administered at least 1 week before randomisation.

6. Patients receiving treatment with chloroquine or derivatives within 8 weeks before enrolment or during the study.
7. Patients receiving treatment with strong CYP3A4 inhibitors or inducers (Appendix 4 – Inhibitors and Inducers of CYP3A4).
8. Viral illness (other than COVID-19) requiring therapy, except for patients with treated and adequately controlled (undetectable) human immunodeficiency virus infection.
9. Patients with uncontrolled known primary or secondary immunodeficiency, including chronic treatment with glucocorticoids (i.e., prednisone at a daily dose of >10 mg for >1 month, or another glucocorticoid at equipotent dose).
10. Any of the following cardiac conditions or risk factors:
    - Sinus bradycardia (<50 beats/min), sinus nodal dysfunction (sick sinus disease), atrio-ventricular block of any degree (PR >200 msec), or any other bradyarrhythmia (<50 beats/min), except for patients with permanent pacemakers;
    - Cardiac infarction, cardiac surgery or cardiac insufficiency episode within the last 6 months;
    - Known abnormal value of left ventricular ejection fraction (LVEF <LLN), unless documented confirmation of recovery (LVEF >LLN) in the previous month;
    - QT interval corrected using Fridericia's formula (QTcF) >450 msec for males or >470 msec for females;
    - History of known congenital or acquired QT prolongation;
    - Uncorrected hypokalaemia, hypocalcaemia (adjusted), and/or hypomagnesemia at screening;
    - Troponin test performed at local laboratory >1.5 x ULN; or
    - Need for an unreplaceable drug that prolongs QT and it is clearly associated with a known risk for torsades de pointes (TdP) (Appendix 8a - Lists of Drugs That Prolong the QT Interval and/or Induce Torsades de Pointes Ventricular Arrhythmia); in case of being already on treatment with these aforementioned drugs, a minimum of 4 half-lives of the drug is required before replacement (if feasible).
11. Hypersensitivity to the active ingredient or any of the excipients (mannitol, macrogolglycerol hydroxystearate, and ethanol) or patients for whom dexamethasone, antihistamine H1/H2 or antiserotoninergic agents are contraindicated.

12. Females who are pregnant (negative serum or urine pregnancy test required for all females of child-bearing potential at screening) or breast feeding.
13. Females and males with partners of child-bearing potential (females who are not surgically sterile or postmenopausal defined as amenorrhoea for >12 months) who are not using at least 1 protocol-specified method of contraception.
14. Any other clinically significant medical condition (including major surgery within the last 3 weeks before screening) or laboratory abnormality that, in the opinion of the investigator, would jeopardise the safety of the patient or potentially impact on patient compliance or the safety/efficacy observations in the study.

4.3. Disease Diagnostic Criteria

Patients with a documented diagnosis of SARS-CoV-2 infection performed by a local laboratory through either qualitative PCR, antigen test, or any other validated method approved by the local health authority, from appropriate biological samples collected no more than 72 hours before study treatment on Day 1, meeting category 5 on the 11-point WHO Clinical Progression Scale (Appendix 13 - World Health Organization (WHO) Clinical Progression Scale), and symptoms onset not beyond 14 days before initiation of study treatment, are required for enrolment. In the case that the patient had experienced more than one COVID-19 episodes, onset of symptoms should be referred to the most recent one.

4.4. Discontinuation Criteria

4.4.1. Screen Failures

Screen failures are defined as patients who consent to participate in the clinical study but are not subsequently enrolled in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure patients to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAEs related to the study participation. Rescreening is not allowed in this study.

4.4.2. Early Discontinuation of Study

Patients may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, or administrative reasons. If the patient withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent. Additionally, patients may request destruction of any samples taken and not tested, and the investigator must document this in the site study records. Refer to the Schedule of Assessments (Appendix 5) for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

Additional reasons for discontinuation can include, but are not limited to:

- Administration of treatments not allowed in the protocol (see Section 6.1)

- Adverse event
- Lack of efficacy
- Investigator's decision
- Patient refusal as reason for discontinuation

All cases of treatment discontinuation must be documented (date and reason) in the electronic case report form (eCRF). A subject may stop the study treatment but desirably should continue to the end of study.

Patients who receive at least 1 dose of plitidepsin and withdraw from the study will be followed for at least 31 days (±3 days) after administration of the last dose of plitidepsin to follow-up on possible open TEAEs at withdrawal or for potential late-onset treatment-related TEAEs.

Cardiac Changes (e.g., QTc)

If a clinically significant finding is identified including, but not limited to, changes from baseline in QTcF after enrolment, the investigator or qualified designee will determine if the patient can continue in the study and if any change in patient management is needed. This review of the triplicate 12-lead ECGs printed at the time of collection must be documented. Any new clinically relevant finding should be reported as an AE.

A patient who meets either bulleted criterion based on the average of triplicate 12-lead ECG readings will be withdrawn from the treatment:

- Average QTc >500 msec
- Average change from baseline QTc >60 msec
- TdP
- Polymorphic ventricular tachycardia
- Signs/symptoms of serious arrhytmia

4.4.3. Lost to Follow-up

A patient will be considered lost to follow-up if he/she repeatedly fails to return for scheduled visits on Days 8 (±1 day) and 31 (±3 days), and is not reachable for Day 15 (±1 day) call or visit, and is unable to be contacted by the study site.

The following actions must be taken if a patient fails to return to the clinic for a required study visit or fails to answer the phone call for Day 15 (±1 day):

- The site must attempt to contact the patient and reschedule the missed visit as soon as possible, counsel the patient on the importance of maintaining the assigned visit schedule, and ascertain whether or not the patient wishes to and/or should continue in the study.
- In cases in which the patient is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the patient (where possible, 3 telephone calls and, if necessary, a certified letter to the patient's last known mailing address or

local equivalent methods). These contact attempts should be documented in the patient's medical record.

Should the patient continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of loss to follow-up.

4.4.4. Replacement Procedures

No formal replacement of patients is foreseen. As the primary analysis of the study will be conducted in the Intent-to-Treat (ITT) population (see Section 8.2.2), the accrual will continue until 609 patients in the ITT population have been recruited.

4.4.5. Follow-up of Patients Prematurely Discontinued From the Study Treatment Regimen or Withdrawn From Study

Due to continued scientific importance of patient data even if study treatment is discontinued early, patients who withdraw from the study will be asked to complete all subsequent study procedures (follow-up/end of study visit), as described in the Schedule of Assessments (Appendix 5). Patients who are no longer hospitalised may complete their assessments by telephone or other means of remote communication.

Treatment-related adverse events, treatment-emergent adverse events of special interest, and SAEs that are ongoing at the time of discontinuation, or that develop before the protocol-specified Day 31 (±3 days) follow-up visit, will be followed until resolution or stabilisation.

Steps will be taken by the sites to ascertain vital status in all randomised patients (e.g., with a vital records search) up through Day 31.

4.5. Stopping Rules

The study will consist of approximately 609 patients included in the ITT population and randomised as 1:1:1 to 3 arms (plitidepsin 1.5 mg, plitidepsin 2.5 mg, and control) with 203 patients in each arm. A futility analysis for efficacy/safety will be performed when 33% of events (sustained withdrawal of supplementary oxygen) have been reached. The rho family of beta-spending functions (with $\rho=1.18$) will be used for the calculation of the stopping boundaries for futility in order to control type II error. The stopping boundaries for the futility analysis will be calculated with the actual number of events available in the ITT population at that moment; if the number of events at the futility analysis is 116 in a pair comparison, the beta-spending corrected p-value to reject the alternative hypothesis should be higher or equal than 0.4199 and this p-value is associated with a $HR \leq 1.0382$.

If the recommendation of the IDMC is to discontinue one of the plitidepsin dosing arms at this futility analysis following the stopping rules, the study will continue from that point on a 1:1 randomisation fashion until the completion of the remaining treatment arms (203 patients per arm and 353 events of sustained withdrawal of supplementary oxygen in total necessary to perform the final analysis). The patients still ongoing in the dropped arm would continue to be followed up as per protocol.

Study Termination

The sponsor reserves the right to close a study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study site closure visit has been performed. Reasons for study termination may include, but are not limited to:

- Adverse events unknown to date (i.e., not previously reported in any similar investigational study drug trial with respect to their nature, severity, and/or duration).
- Increased frequency and/or severity and/or duration of known, anticipated, or previously reported AEs (this may also apply to AEs defined at enrolment as baseline signs and symptoms).
- Medical or ethical reasons affecting the continued performance of the study.
- Difficulties in the recruitment of patients.
- Cancellation of drug development.

The investigator may initiate study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination. Reasons for the early closure of a study site by the sponsor or investigator may include, but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) or local health authorities, the sponsor's procedures, or Good Clinical Practice (GCP) guidelines.
- Inadequate recruitment of participants by the investigator.

5. STUDY TREATMENTS

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study patient according to the study protocol. Study treatments for this study are summarised in the section below.

5.1. Treatments Administered

INN name	**Plitidepsin**
Pharmaceutical form	Powder and solvent for concentrate for solution of infusion
Formulation	Plitidepsin 2 mg powder is provided as a sterile, preservative-free, and white to off-white lyophilised powder/cake comprising 2 mg plitidepsin and [redacted] mannitol in a single-dose, 10 mL clear type 1 glass vial. Solvent for plitidepsin is provided as a sterile, preservative-free, clear, slightly viscous aqueous liquid (4 mL) containing [redacted] macrogolglycerol ricinoleate and [redacted] ethanol in a single-dose type 1 clear glass ampoule. For administration, vial contents are reconstituted by addition of 4 mL of solvent for plitidepsin to obtain a slightly yellowish solution containing 0.5 mg/mL plitidepsin with mannitol, macrogolglycerol ricinoleate and ethanol excipients. The required amount of plitidepsin reconstituted solution is added to bag containing 0.9% sodium chloride or 5% glucose for IV injection and administered as an IV infusion over 60 minutes.
Packaging and labelling	Packaging and labelling will be in accordance with applicable local regulatory requirements and applicable Good Manufacturing Practice (GMP) guidelines.
Storage	Plitidepsin should be stored refrigerated between 2ºC and 8ºC and the vials should be kept in the outer carton to protect them from light. Excursions in temperature between 0°C and 15°C that may be experienced during shipping and distribution may be allowed. Do not freeze. The drug in these conditions is stable for 60 months. The plitidepsin solution for infusion should be administered within 6 hours of reconstitution (time of administration to the patient included) if stored at room temperature and under ambient lighting. If storage is required prior to administration, then solutions should be stored refrigerated and protected from light and should be used within 24 hours of reconstitution. Plitidepsin is a cytotoxic medicinal product. Any unused medicinal product or waste material should be disposed of in accordance with local requirements.
Manufacturer	Pharma Mar S.A., Madrid, Spain
INN Name	**Dexamethasone** **Remdesivir** **Favipiravir**
Detailed information about the formulation, posology, packaging and labelling, storage, and manufacturer is provided in the current country-specific product information. The SmPC and/or leaflet provides detailed product information for investigators in the European Union and/or in other regions. Packaging and labelling will be in accordance with applicable local regulatory requirements and applicable GMP guidelines.	

Abbreviations: INN = international nonproprietary name; SmPC = summary of product characteristics

All patients will receive dexamethasone phosphate 8 mg/day IV (equivalent to 6.6 mg dexamethasone base) on Days 1 to 3 (administered as a premedication in plitidepsin arms), followed by dexamethasone phosphate 7.2 mg/day (equivalent to 6 mg/day dexamethasone base) PO/IV from Day 4 and up to a total cumulative dose of 60 mg of dexamethasone base (as per physician judgement according to patient clinical condition and evolution).

Additionally, in accordance with local treatment guidelines, patients randomised to the control arm may receive a regulatory-approved antiviral treatment, such as remdesivir (200 mg IV on Day 1 followed by 100 mg/day IV on Days 2 to 5) or favipiravir (1600 mg BID PO on Day 1, followed by 600 mg BID PO daily for 2 to 5 days).

Prophylactic Medications for Plitidepsin

To mitigate plitidepsin infusion reaction, administration of the following IV premedications should be ideally completed 20 to 30 minutes before initiating the plitidepsin infusion and, exceptionally, up to 2 h before plitidepsin infusion start:

- Palonosetron 0.25 mg IV. Tropisetron 5 mg IV could be considered if palonosetron is not available.
- Diphenhydramine hydrochloride 25 mg IV (or equivalent such as dexchlorpheniramine maleate 5 mg).
- Ranitidine 50 mg IV (or equivalent, such as famotidine 20 mg IV).
- Dexamethasone phosphate 8 mg IV (equivalent to 6.6 mg dexamethasone base).

Additionally, to mitigate plitidepsin-induced nausea and vomiting, on Days 4 and 5 patients treated with plitidepsin should receive tropisetron 5 mg PO/IV if tropisetron 5 mg IV was administered on Days 1, 2, and 3.

Preparation, Storage, Handling, and Accountability

Plitidepsin will be supplied by the sponsor along with the (batch/lot) numbers and certificates of analysis. Commercially available formulations of dexamethasone and remdesivir, favipiravir, or other regulatory-approved antiviral treatment will be provided, as appropriate. A certificate of release authorised by a qualified person in the European Union will also be issued for the investigational medicinal product (IMP). One vial of plitidepsin for injection, 2 mg, and 1 ampoule of solvent for plitidepsin, 4 mL, are packaged in a carton, which is required for light protection. Plitidepsin for injection is provided in vials containing 2 mg plitidepsin powder. For administration, vial contents are reconstituted by addition of 4 mL of solvent for plitidepsin to obtain a slightly yellowish solution containing 0.5 mg/mL plitidepsin with mannitol, macrogolglycerol ricinoleate and ethanol excipients. The required amount of plitidepsin-reconstituted solution is added to bag containing 0.9% sodium chloride or 5% glucose for IV injection and administered as an IV infusion over 60 minutes.

Plitidepsin vials should be stored refrigerated between 2°C and 8°C. Excursions in temperature between 0°C and 15°C that may be experienced during shipping and distribution may be allowed. Do not freeze.

The pharmacist at each investigational site will be responsible for management of the IMP (plitidepsin, dexamethasone, and any regulatory.approved antiviral treatment, such as remdesivir or favipiravir). The pharmacist will inventory and acknowledge receipt of all shipments of IMP, which must be kept in a locked area with restricted access. The IMP must be stored and handled in accordance with the manufacturer's instructions and temperature traceability is required. The pharmacist will keep accurate reconstitution and dilution records of the quantities (and batch numbers) of IMP dispensed and used for each patient. Periodically, the sponsor's study monitor will check the reconstitution, dilution, supplies of IMP held by the pharmacist to verify accountability of all IMP. All delivery records must be reconciled with usage records. It is essential that all IMP be accounted for by the site's pharmacist and that discrepancies are explained and documented.

At the conclusion of the study, after final accountability has been reviewed and reconciled by the sponsor, all unused IMP and all medication containers will be either returned to the sponsor or, at the written direction of the sponsor, destroyed by the investigational site. In the case of destruction, a certificate of destruction will be provided by the pharmacist to the sponsor. It is essential that all study drugs would be accounted for by the site's pharmacist and that discrepancies are explained and documented.

Returned non-used study treatment should not be re-dispensed to the patients.

5.2. Method of Treatment Assignment

All patients should be recorded into the eCRF from the date of the ICF signature. Patients who meet the eligibility criteria (see Section 4.1 and Section 4.2) will be randomised through IWRS registration. The system will assign a unique random number and specified treatment arm for each patient.

Patients will be randomised 1:1:1 to each of the 3 treatment arms. Randomisation will be stratified as:

a) Geographical Region (Europe *versus* Rest of the World);
b) Charlson Comorbidity Index (0-1 *versus* >1) (Appendix 11 - Age-adjusted Charlson Index); and
c) Barthel Indez (≥90 *versus* <90) (Appendix 7 - Barthel Index for Functional Assessment).

Central randomisation will be implemented on Days 0 to 1 before the first infusion of study drug using stratified permuted blocks to balance groups for stratification factors. Patients will be assigned to each arm at a 1:1:1 ratio. Screening and randomisation should take place within 2 consecutive days or on the same day.

5.2.1. Dose Modification

Dose reductions will not be allowed for plitidepsin. If plitidepsin dose reduction is needed, the patient should be discontinued from treatment and all visit assessments completed.

5.3. Blinding

The WHO R&D BluePrint notes that "In order to minimise bias related to patient selection, retention, co-interventions, treatment and outcome assessment, blinding to all involved in a trial (patients and family, clinician team, trial personnel, etc.) is preferred, especially if the primary endpoint may be sensitive or responsive to decisions of clinical judgement which could be subconsciously influenced by knowledge of treatment allocation. However, blinding may not always be possible, and we recognise the operational difficulties associated with the administration of drug and premedication regimens, preparation of placebos and timelines necessary to initiate experimental research during an epidemic. Blinding will be considered depending upon the interventions used, and when appropriate, for as long as possible, for patients, family members, clinical team members, trial team personnel, outcome assessors and analysts; acknowledging that this may not be possible for some roles at the point-of-care of patients."

5.4. Treatment Compliance

All study drugs will be administered at the site-by-site personnel. The sponsor retains the right to require the withdrawal of any patient who violates the protocol.

6. CONCOMITANT THERAPIES AND OTHER RESTRICTIONS

6.1. Concomitant Therapy

Concomitant medications and treatments will be recorded for all patients from 28 days before the start of study treatment and through study Day 31 (±3 days). All COVID-19 vaccinations should be recorded, regardless of when they were given, until the end of study. All concomitant medications should be recorded in the eCRF including supportive care drugs (e.g., prophylaxis for infusion reactions and antiemetic treatment and prophylaxis), the drugs used to treat AEs or chronic diseases, and non-drug supportive interventions (e.g., transfusions). Concomitant medications should be reviewed at each protocol-specified visit and any prohibited treatments (as described below) should be discussed with the patient and appropriately managed.

6.1.1. Allowed Medications

- Protocol-specified prophylaxis for plitidepsin infusion-related reactions.
- Protocol-specified antiemetic prophylaxis for plitidepsin.
- Supportive treatments for symptoms/AEs (e.g., antiemetics, analgesic, anti-inflammatory), including the use of locally approved agents blocking inflammatory cytokines signaling (e.g., tocilizumab, anakinra), as per local guidelines. Duration and doses of dexamethasone may be adjusted in case of clinical deterioration as per physician's judgement. Recommended medications to treat infusion-related reactions, hypersensitivity reactions, and other drug-related events are described in Section 6.2.
- Standard treatments for concomitant conditions, including transfusion support, anticoagulants, insulin, and antibiotics.
- Anticoagulants (preferably low-dose low-molecular weight heparin) are allowed; as clinically indicated, appropriate monitoring of prothrombin time (PT) should be performed, if applicable. Patients who develop venous thromboembolic events during study requiring therapeutic levels of anticoagulants must be monitored closely for their platelet counts.
- SARS-CoV-2 vaccination is allowed.

Throughout the study, investigators will be allowed to administer medication for supportive treatment of symptoms and AEs, as well as standard treatments for concomitant conditions.

6.1.2. Prohibited Medications:

- Prior (within 8 weeks prior to enrolment) or concomitant administration of chloroquine phosphate or hydroxychloroquine sulfate during the study
- The use of unregistered and/or experimental medicinal products, and the off-label use of any other drug for the primary treatment of SARS-CoV-2 infection is not permitted throughout the study
- Any other concomitant antiviral treatment for COVID-19 while receiving study treatment in any of the study arms.

- Treatment with convalescent plasma or monoclonal antibodies against SARS-CoV-2 within 2 weeks before enrolment, unless objective evidence of clinical deterioration documented at least 1 week after the end of such therapy.
- Concomitant use of drugs susceptible to induce QT prolongation within 48 hours before enrolment and during the study (Appendix 8a - Lists of Drugs That Prolong the QT Interval and/or Induce Torsades de Pointes Ventricular Arrhythmia).
- Concomitant administration of treatment with strong CYP3A4 inhibitors or inducers (Appendix 4 - Inhibitors and Inducers of CYP3A4).

6.1.3. Drug interactions With Plitidepsin:

In vitro studies indicated that CYP3A4 is the major enzyme involved in plitidepsin metabolism, followed by CYP2A6 and CYP2E1. Although a PK population analysis of plitidepsin in 283 cancer patients showed that concomitant administration of CYP3A4 inhibitors and inducers did not affect exposure to plitidepsin, it cannot be ruled out that they modify the efficacy and/or increase the probability of side effects associated with plitidepsin.

Accordingly:

- Strong inhibitors of CYP3A4 (Appendix 4 - Inhibitors and Inducers of CYP3A4), including clarithromycin, itraconazole, nefazodone, telithromycin, voriconazole, and grapefruit juice, should not be coadministered with plitidepsin unless there is no therapeutic alternative. Administration should be discontinued before starting plitidepsin treatment and should be withheld until 24 hours after the last dose of plitidepsin.
- Moderate inhibitors of CYP3A4 (Appendix 4 - Inhibitors and Inducers of CYP3A4), including aprepitant, diltiazem, erythromycin, fluconazole, and verapamil, should be used with caution as increased exposure to plitidepsin and increased potential for side effects cannot be ruled out.
- Strong inducers of CYP3A4 (Appendix 4 - Inhibitors and Inducers of CYP3A4), including anticonvulsants (phenytoin or carbamazepine), rifampicin, and St. John's wort, should not be coadministered with plitidepsin unless there is no therapeutic alternative. Administration should be discontinued before starting plitidepsin treatment and should be withheld until 24 hours after the last dose of plitidepsin.
- Moderate inducers of CYP3A4 (Appendix 4 - Inhibitors and Inducers of CYP3A4), including bosentan, phenobarbital, and primidone should be used with caution, as a reduction in exposure to plitidepsin cannot be excluded.

6.2. Supportive Care

All study participants, whether randomised to plitidepsin or control arms, should receive appropriate supportive care measures as deemed necessary by the treating investigator. In particular, patients randomised to plitidepsin arms may receive the following treatments:

- Infusion Reactions/Hypersensitivity Reactions:
  - If a mild/moderate (Grade 1 to 2) reaction, such as allergic symptoms, stop plitidepsin infusion immediately and continuously monitor vital signs and pulse oximetry. Administer dexamethasone 4 mg IV. If symptoms persist after administering dexamethasone, administer diphenhydramine 50 mg IV (or equivalent) and hydrocortisone 100 mg IV (or equivalent).
  - Re-evaluate symptoms after 30 minutes. If symptoms have improved or normalised, resume the plitidepsin infusion at a rate 1/3 of the initial infusion rate during the first hour. Continuously monitor vital signs and pulse oximetry during the infusion. If the infusion is being well tolerated, the infusion rate can be increased.
  - Any subsequent infusions of plitidepsin also should be initiated at 1/3 the normal rate with continuous monitoring of vital signs and pulse oximetry during the first hour. If no signs of infusion-related reaction are observed, the infusion rate can be increased.
  - If symptoms have not improved or normalised within 30 minutes after administration of dexamethasone, diphenhydramine (or equivalent), and hydrocortisone (or equivalent), administer additional antihistamine and/or corticosteroids until resolution and permanently discontinue plitidepsin treatment.
  - If severe/life-threatening (Grade 3 to 4) reaction, stop the plitidepsin infusion immediately and consider treatment with bronchodilators and oxygen. If clinically indicated, treat with diphenhydramine 50 mg IV (or equivalent), hydrocortisone 100 mg IV (or equivalent), and epinephrine (adrenaline) 100 to 200 µg IV. Permanently discontinue plitidepsin treatment.
- Diarrhoea: All patients who experience diarrhoea should be advised to drink liberal quantities of clear fluids. If sufficient oral intake is not feasible, fluid and electrolytes should be substituted via IV infusion.

  For patients with uncomplicated mild to moderate diarrhoea, loperamide (4 mg initial dose followed by 2 mg every 4 hours) might be considered, as per local guidelines, except if contraindication.

  Patients with persistent mild to moderate (>48 hours after start of loperamide) who have any one of the following added risk factors: moderate to severe cramping, NCI-CTCAE Grade ≥2 nausea/vomiting, decreased performance status, fever, sepsis, neutropenia, frank bleeding, or dehydration, should be evaluated further and monitored closely. These patients should be classified as "complicated" and may require stool work-up (evaluation for blood, faecal leucocytes, *C difficile*, *Salmonella*, *E coli*, *Campylobacter*, and infectious colitis), complete blood count, and electrolyte profile, together with more aggressive management. Any patient with NCI-CTCAE Grade 3 or 4 diarrhoea would be classified as "complicated" and require aggressive management.

  Aggressive management consist in fluid and electrolytes repletion as needed and second-line antidiarrhoeal agent such as subcutaneous (SC) octreotide (100- to 150-µg starting dose, with dose escalation as needed) or other second-line agents (e.g., oral budesonide or tincture of opium).

- Nausea/Vomiting: Protocol-specified- prophylaxis for emesis should be administered, as specified in Section 5.1 (prophylactic medications), and nausea/vomiting that occurs despite this prophylaxis should be treated aggressively. Patients should be strongly encouraged to maintain liberal fluid intake. If sufficient oral intake is not feasible, fluid and electrolytes should be substituted via IV infusion.

7. STUDY ASSESSMENTS AND PROCEDURES

Study procedures and their timing are summarised in the Schedule of Assessments (Appendix 5). As protocol waivers or exemptions are not allowed, with the exception of immediate safety concerns, these should be discussed with the sponsor immediately upon occurrence or awareness to determine if the patient should continue or discontinue the study drug. Adherence to the study design requirements, including those specified in the Schedule of Assessments, is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential patients meet all eligibility criteria. The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure (Section 4.4.1), as applicable. Procedures conducted as part of the patient's routine clinical management (e.g., blood count) and obtained before signing the ICF may be utilised for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the timeframe defined in the Schedule of Assessments.

Every effort should be made to ensure that protocol-required tests and procedures are completed. When a protocol-required test or procedure cannot be performed, the investigator will document the reason for this and any corrective and preventive actions the investigator has taken to ensure that normal processes are adhered to as soon as possible. The investigator will inform the study team of these incidents in a timely manner.

The maximum amount of blood collected from each patient over the duration of the study will not exceed 101.1 mL for the main study and additional 22 mL for the PK substudy. Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

7.1. Efficacy Assessments

7.1.1. Test for COVID-19

Quantitative PCR to assess viral load on Day 1 before initiation of treatment and on Day 8 (±1 day) (see Schedule of Assessments [Appendix 5], will be performed by a central laboratory. Based on these assessments, changes in viral load from Day 1 to Day 8 will be calculated for each patient. The proportion of patients with undetectable SARS-CoV-2 on Day 8 will also be assessed.

Details regarding the collection, processing, storage, and shipment of oro-nasopharyngeal samples for qPCR testing will be provided to each investigational site before initiation of the study in the form of a laboratory manual. All oro-nasopharyngeal samples for qPCR must be processed and shipped as specified in the laboratory manual to maintain sample integrity. Any deviations from the processing steps (e.g., sample collection and processing steps, interim storage, or shipping conditions), including any actions taken, must be documented and reported to the sponsor. On a case-by-case basis, the sponsor may make a determination as to whether sample integrity has been compromised. Any deviation from the specified sample handling procedure that results in compromised sample integrity will be considered a protocol deviation.

Samples will be analysed using validated analytical methods, as approved by the sponsor.

7.1.2. Chest Imaging

Chest radiograph abnormalities will be detected by chest radiographs (X-ray) or chest CT scan on Day 1, 8 (±1 day), and 31 (±3 days); for each patient, the same assessment technique is to be used for each timepoint. Chest imaging performed within 48 hours of Day 1 is accepted and does not have to be repeated. Images will be reviewed for the presence of pulmonary infiltrates, pleural fluid, atelectasis, pulmonary oedema, and other findings.

7.1.3. Clinical and Functional Status

Clinical status (including the need for oxygen supplementation) will be assessed at least once a day, or whenever it changes, while hospitalised and on Days 8 (±1 day), 15 (±1 day), and 31 (±3 days) using the 11-point WHO Clinical Progression Scale (Appendix 13 – World Health Organization (WHO) Clinical Progression Scale).

The pre-baseline (i.e., > 1 month prior to screening) functional status should be recorded, in addition to Day 1 prior to initiating study treatment, at hospital discharge, and on Days 8 (±1 day), 15 (±1 day), and 31 (±3 days); functional status will be assessed by the Barthel index (Appendix 7 – Barthel Index for Functional Assessment).

7.1.4. Other Efficacy Assessments

The study day (from Day 1 to Day 31 (±3 days)) on which the following events occurred will be recorded:

- Initiation of supplemental oxygen (of any type)
- Discontinuation of supplemental oxygen (of any type)
- Initiation of respiratory support
- Discontinuation of respiratory support
- Initiation of antivirals or immune-modulating drugs
- Discotinuation of antivirals or immune-modulating drugs
- ICU admission
- ICU discharge
- Hospital re-admission for COVID-19 signs or symptoms
- Hospital discharge
- Death

Additionally, the following information will be gathered for each patient on Days 4, 8 (±1 day), 15 (±1 day), and 31 (±3 days) (yes/no):

- Receiving supplementary oxygen therapy
- Receiving high-flow oxygen
- Receiving non-invasive mechanical ventilation
- Receiving invasive mechanical ventilation or ECMO
- Requiring hospital admission related to COVID-19
- Requiring ICU admission
- Receiving subsequent antiviral therapies or immunomodulatory drugs

- Nosocomial infection
- Death

Based on these data, secondary endpoints for percentages of patients requiring oxygen therapy or different types of ventilation will be calculated and the time (in days) to sustained hospital discharge or withdrawal of supplemental oxygen therapy, percentages of patients with nosocomial infection or receiving subsequent antiviral or immunomodulatory therapies, hospitalisation in ICU, and hospitalisation will be measured.

Laboratory-based efficacy assessments that are to be performed during the study include:

- Proinflamatory biomarkers: (CRP, LDH, ferritin, IL-1β, IL-6, IL-10, and TNFα) in each study arm from baseline (Day 1 prior to start of the study treatment) to Days 2, 3, 4, 8 (±1 day), and 31 (±3 days).
- Serological test (IgG) on Days 1 and 31 (±3 days).

7.2. Safety and Tolerability Assessments

Safety will be assessed throughout the study through clinical and laboratory safety evaluations. These include medical history at screening, physical examinations, vital sign measurements, saturation of oxygen (SpO_2) by pulse oximetry (or arterial blood gas analyses [PaO_2]), and its respective FiO_2 (Appendix 12 - Imputation of FiO_2 from oxygen flow values. High-flow oxygen therapy), triplicate 12-lead ECGs, laboratory tests (haematology, coagulation, and chemistry), clinical AE monitoring, and verification of concomitant treatments.

7.2.1. Adverse Events

Adverse event definitions and assignment of severity and causality are detailed in Appendix 1. All adverse events (AE) and adverse reactions (ARs), based on clinical signs and symptoms and laboratory measurements, will be measured daily until Day 31 (±3 days), except treatment-related adverse events, treatment-emergent adverse events of special interest, and SAES that will be measured daily until resolution or stabilisation to at least Grade 1, or to an acceptable level according to the investigator and the sponsor of his/her designated representative.

Adverse events will be elicited from the patient (or, when appropriate, from a caregiver, surrogate, or the patient's legally authorised representative) by the study site staff using a non-leading question such as "How are you feeling today?" or "Have you had any health concerns since your last visit?"

The investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or the study, or that caused the patient to discontinue the study treatment (see Section 4.4.2).

Clinical Signs and Symptoms: AEs, including worsening of baseline signs and symptoms of disease observed by the investigator (preferably by the same physician for a same patient) or reported by patients to study nurses will be recorded and graded according to NCI-CTCAE version 5.0, accessible via internet

(http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf). All events, including those considered not related to the study drugs, must be reported in the eCRF.

The following AEs will be considered and monitored as AEs of special interest: musculoskeletal disorders, CPK increases, and rhabdomyolysis; hypersensitivity reactions, cardiac events and transaminase elevations, and hepatobiliary disorders.

Laboratory Abnormalities: Laboratory abnormalities will be recorded and graded according to NCI-CTCAE version 5.0, accessible via internet (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf). Laboratory abnormalities that are of clinical significance will be recorded in Adverse Event eCRF.

Laboratory abnormalities for which NCI-CTCAE grading is not available will be classified as the fold below the lower limit of normal (<LLN) or fold above the ULN.

7.2.2. Reporting Serious Adverse Events

All the SAEs, regardless of the causality assessment, occurring after signing of ICF until Day 31 (±3 days), will be reported. In addition, SAEs determined by the investigator to be related to the study drug, that have been identified after completion of the study will also be reported directly to the drug manufacturer as described below.

The SAEs occurring during the screening phase and after off-study will be reported using a paper "SAE form" that must be forwarded as mentioned below always within 24 hours to the Pharma Mar S.A. Pharmacovigilance Department by fax or email.

All SAEs, occurring from the date of initiating the study treatment (Day 1) until Day 31 (±3 days) and regardless of study drug relationship, must be reported immediately, and always within 24 hours to the Pharma Mar S.A. Pharmacovigilance Department electronically by completing the applicable eCRF section. Only in situations of electronic system failure or pregnancy exposure during the clinical trial, the SAEs can be reported using paper on a SAE form by fax (+34 91 846 6004) or email (AEincoming@pharmamar.com). Out of office hours (Greenwich Meridian Time [GMT]), assistance on SAE reporting can be obtained by calling the Pharmacovigilance Department at +34 681 263 592. In case of electronic system failure or pregnancy exposure during the clinical trial, SAEs initially reported by alternative methods (not electronically), must be followed by a completed electronic SAE reporting on eCRF from the investigational staff within 1 working day.

All SAEs, regardless of the relationship with the study drug, must be followed until the event or its sequelae resolves or stabilises to at least Grade 1, or to an acceptable level according to the investigator and the sponsor or his/her designated representative.

7.2.3. Pregnancy

For female patients of child-bearing potential, a serum or urine pregnancy test, with sensitivity of at least 25 mIU/mL, and assayed in a certified laboratory, will be performed at screening.

Following a negative pregnancy test result at screening, appropriate contraception must be commenced before the patient may receive study drugs.

Additional pregnancy tests will be performed whenever 1 menstrual cycle is missed or when potential pregnancy is otherwise suspected. In the case of a positive confirmed pregnancy, the patient will be withdrawn immediately from administration of study drugs, but may remain in the study for safety and efficacy follow-up.

Fertile male patients and female patients who are of child-bearing potential, who are, in the opinion of the investigator, sexually active and at risk of pregnancy with their partner(s), will need to affirm that they meet the criteria for use of highly effective contraception methods throughout the study and for at least 6 months after the last dose of plitidepsin. Patients in the control arm must use effective contraception during the time indicated in the approved product information (SmPC or leaflet). If no information is available in the approved product information, patients in the control arm must use effective contraception for at least one week after the study completion or the time indicated based on the investigator's discretion. The investigator, or designated subinvestigator, will discuss with the patient the need to use effective contraception methods consistently and correctly and document such conversation in the patient's chart. In addition, the investigator will instruct the patient to call immediately if contraception methods are discontinued or if pregnancy is known or suspected in the patient or the patient's partner.

7.2.4. Clinical Laboratory Evaluations

Blood samples for laboratory safety assessments, including haematology (RBC, haemoglobin, haematocrit, WBC with differential, platelet count), coagulation (D-dimer), serum chemistry (ALT, AST, alkaline phosphatase, GGT, lactate dehydrogenase [LDH], total bilirubin, direct bilirubin, glucose [fasting], sodium, potassium, calcium (albumin adjusted calculation), magnesium, BUN, creatinine, calculated creatine clearance [Cockcroft-Gault equation], CPK, albumin, amylase, lipase, procalcitonin, troponin [troponin I or T according to local practice for screening and troponin T [high sensitivity] by central laboratory for on-study tests], and N-terminal pro-B-type natriuretic peptide [NT-pro-BNP], serological SARS-CoV-2 testing (immunoglobulin [Ig]G), and immunology (CRP, ferritin, IL-1β, IL-6, IL-10, TNFα) will be drawn at the timepoints described in the Schedule of Assessments (Appendix 5). Testing to be performed by local laboratory at screening and by central laboratory for on-study tests at Day 1 prior to initiation of study treatment, once daily during hospitalisation, and at follow-up visits on Days 8 (±1 day) and 31 (±3 days), except, troponin T (high sensitivity), and NT-pro-BNP that will be measured on Day 1, Day 8 (±1 day), and Day 31 (±3 days). Tests performed within 24 hours of screening do not have to be repeated.
Details regarding the collection, processing, storage and shipment of blood samples for laboratory safety assessments will be provided to each investigational site before initiation of the study in the form of a laboratory manual. All blood samples must be processed and shipped as specified in the laboratory manual to maintain sample integrity. Any deviations from the processing steps (e.g., sample collection and processing steps, interim storage or shipping conditions), including any actions taken, must be documented and reported to the sponsor. On a case-by-case basis, the sponsor may make a determination as to whether sample integrity has

been compromised. Any deviation from the specified sample handling procedure that results in compromised sample integrity will be considered a protocol deviation.

7.2.5. Vital Signs, Physical Examination, and ECGs

Patients will have a physical examination that includes major body systems and measurements of body weight, height, body temperature, sitting blood pressure, heart rate, respiratory rate, and PaO_2 (obtained either by arterial blood gas analyses or from saturation of oxygen (SpO_2) by pulse oximetry, and its respective FiO_2 (Appendix 12 Imputation of FiO_2 from oxygen flow values. High-flow oxygen therapy), at the timepoints specified in the Schedule of Assessments (Appendix 5). Additionally, height will be measured at screening. To estimate the PaO_2/FiO_2 ratio, the required equation would be used for imputing PaO_2 from SpO2 (Appendix 9 – Imputation of PaO_2 from SpO_2), and the information on the oxygen delivery devices and oxygen flow prescribed would be utilised for imputation of FiO_2.

Triplicate 12-lead (with a 10-second rhythm strip) tracings will be used for all ECGs, taken at the timepoints described in the Schedule of Assessments (Appendix 5). Preferably, the machine used has a capacity to automatically calculate the standard intervals. At each test point, QTcF prolongation to be assessed at screening (defined as the visit with less than 24 hours of difference before the first infusion of the study drug) and on Days 1, 3, and 31 (±3 days). If the mean QTc is prolonged (>500 msec, ie, CTCAE Grade ≥3) or a change >30 msec from baseline is observed, then the ECGs should be re-evaluated by a specialist at the site for confirmation as soon as the finding is made, including verification that the machine reading is accurate. If manual reading verifies a QTc >500 msec, immediate correction for reversible causes (including electrolyte abnormalities, hypoxia and concomitant medication with drugs with the potential to prolong the QTc interval) should be performed.

Any QTcF values > 500 msec will be flagged as increasing the likelihood of the treatment being proarrhythmic (per ICH Guidance E14).

Each episode of prolongation of the QTc interval will be evaluated by a specialist to determine if it is due to the investigational product(s) or due to other potential precipitating factors (e.g., change in patient clinical condition, effect of concurrent medication, electrolyte disturbance).

If a patient experiences any cardiac or neurologic AEs (especially syncope, dizziness, seizures, or stroke), a 12-lead ECG in triplicate should be obtained at the time of the event.

Clinically significant new findings seen on any ECGs taken after screening should be recorded as AEs.

Additionally, a dedicated QTc substudy will be performed in a subset of approximately 50 patients with ECGs collected over Days 1 to 3 using Holter monitors to assess treatment impact on QTc prolongation. See Appendix 8 for objectives and respective endpoints planned for the QTc substudy. The substudy patients will have blood sampling for pharmacokinetic assessments over Days 1 to 4.

7.3. Pharmacokinetic Analysis

Patients participating in the QTc substudy will have blood sampling for PK assessments over Days 1 to 4. See Appendix 8 (Protocol for QTc Substudy) for details.

7.4. Study Procedures

7.4.1. Prestudy Screening (Days 0 to 1)

Each candidate patient will be examined before starting the study to determine eligibility for participation. Screening assessments normally should be completed within 24 hours before the first administration of study medication. The following evaluations will be performed:

- Informed consent must be obtained before any study specific procedure.
- Verification of eligibility criteria.
- Demographics.
- Charlson Comorbidity Index.
- Medical history.
- Physical examination including body weight, height, vital signs (temperature, blood pressure, heart rate, respiratory rate), PaO_2 (obtained either by arterial blood gas analyses or from saturation of oxygen (SpO_2) by pulse oximetry, and its respective FiO_2 (Appendix 9).
- Clinical status using the 11-point WHO Clinical Progression Scale (Appendix 13).
- Functional status using the Barthel index (Appendix 7) score for the previous month before screening.
- Functional status using the Barthel index score at screening.
- Pregnancy test for females of child-bearing potential.
- Screening symptoms and complaints.
- Adverse events/TEAEs (Appendix 1).
- Concomitant medications.
- Haematology (RBC, haemoglobin, haematocrit, WBC with differential, platelet count) (tests performed within 24 hours of screening do not have to be repeated).
- Serum chemistry (ALT, AST, total bilirubin, sodium, potassium, calcium [albumin adjusted], magnesium, creatinine, calculated creatinine clearance [Cockcroft-Gault equation], CPK, and troponin [I or T according to local practice]) (test performed within the previous 24 hours before screening is accepted and does not have to be repeated).
- Qualitative PCR or antigen test for COVID-19 by local laboratory collected no more than 72 hours before study treatment on Day 1. Record SARS-CoV-2 variant, if available.
- Triplicate 12-lead ECG to assess QTcF interval.

- Screening registration in IWRS.

7.4.2. Evaluations During Study Treatment

7.4.2.1. Days 1 to 4

The following assessments will be completed on Days 1 to 4 unless otherwise stated:

- Randomisation in IWRS (*Day 1 only*).
- Vital signs (temperature, blood pressure, heart rate, respiratory rate) and PaO_2 (obtained either by arterial blood gas analyses or from saturation of oxygen (SpO_2) by pulse oximetry, and its respective FiO_2 just before the start and immediately at the end of the plitidepsin infusion (for patients randomised to plitidepsin arms) and once daily while hospitalised (all arms).
- Physical examination.
- Triplicate 12-lead ECG to assess QTcF interval (*Days 1 and 3 postinfusion*).
- Chest imaging (CT scan or x-ray) (*Day 1 only*) (chest imaging performed within 48 hours of Day 1 is accepted and does not have to be repeated).
- Haematology (RBC, haemoglobin, haematocrit, WBC with differential, platelet count).
- Coagulation (D-dimer).
- Serum chemistry (ALT, AST, alkaline phosphatase, GGT, LDH, total bilirubin, direct bilirubin, glucose [fasting], sodium, potassium, calcium (albumin adjusted calculation), magnesium, BUN, creatinine, calculated creatinine clearance [Cockcroft-Gault equation], CPK, troponin T [high sensitivity; *Day 1 only*], NT-Pro-BNP [*Day 1 only*], albumin, amylase, lipase, and procalcitonin).
- Immunology (CRP, ferritin, IL-1β, IL-6, IL-10, and TNFα).
- Quantitative PCR for COVID-19 viral load (*Day 1 only, prior to initiating study treatment*).
- Serological SARS-CoV-2 testing (IgG) (*Day 1 only*).
- Concomitant medications.
- Adverse events/TEAEs.
- Clinical status, using the 11-point WHO Clinical Progression Scale (Appendix 13).
- Administration of dexamethasone base 6.6 mg/day IV (equivalent to 8 mg dexamethasone phosphate) on Days 1 to 3 (administered as a premedication in plitidepsin arms), followed by 6 mg/day PO/IV (equivalent to 7.2 mg dexamethasone phosphate) from Day 4 and up to a total cumulative dose of 60 mg of dexamethasone base (per physician judgement based on patient clinical condition and evolution).
- Administration of plitidepsin on Days 1 to 3 (*patients randomised to plitidepsin arms*) Administration of plitidepsin will require at least 1.5 hours (±5 mins): 30 minutes for administration of prophylactic medications for infusion reactions (which should be

ideally completed 20 to 30 minutes before plitidepsin infusion start and, exceptionally, up to 2 h pre-infusion of plitidepsin) followed by a 60-minute infusion of plitidepsin. A 30 (±5) minute window is allowed for the plitidepsin administration between each day, and plitidepsin should not be given earlier than 23.5 hours on Days 2 and 3 of previous day plitidepsin administration.

- Administration of remdesivir 200 mg IV on Day 1, followed by 100 mg/day IV on Days 2 to 5 (*per physician decision for patients randomised to control arm*). Administration of favipiravir 1600 mg BID PO on Day 1, followed by 600 mg BID PO daily for 2 to 5 days (*per physician decision for patients randomised to control arm*). Administration of any other regulatory-approved antiviral treatment to follow approved SmPC.

7.4.2.2. Days 5, 6, and 7

The following assessments will be completed on Days 5, 6, and 7 (or until discharged) unless otherwise stated:

- Vital signs (temperature, blood pressure, heart rate, respiratory rate) and PaO_2 (obtained either by arterial blood gas analyses or from saturation of oxygen (SpO_2) by pulse oximetry, and its respective FiO_2 once daily while hospitalised.
- Concomitant medications.
- Adverse events/TEAEs.
- Clinical status, using the 11-point WHO Clinical Progression Scale (highest category that applies) (Appendix 13):

For patients who remain hospitalised after Day 7, the above assessments should be performed once each day until discharge.

Additionally, administration of dexamethasone base will be continued at 6 mg/day PO/IV (equivalent to 7.2 mg dexamethasone phosphate), per physician judgement based on patient clinical condition and evolution. Administration of remdesivir 100 mg/day IV or favipiravir 600 mg BID PO daily may continue until Day 5 per physician's decision for patients randomised to control arm. Administration of any other regulatory-approved antiviral treatment to follow approved SmPC.

7.4.2.3. Days 8 and 31

The following assessments will be performed on Days 8 (±1 day), 31 (±3 days), and study discontinuation unless otherwise stated:

- IWRS registration (*Day 31 and at end of study visit*).
- Vital signs (temperature, blood pressure, heart rate, respiratory rate) and PaO_2 (obtained either by arterial blood gas analyses or from saturation of oxygen (SpO_2) by pulse oximetry, and its respective FiO_2.
- Physical examination.
- Triplicate 12-lead ECG to assess QTcF interval (*Day 31 only and at end of study visit*).

- Haematology (RBC, haemoglobin, haematocrit, WBC with differential, platelet count).
- Coagulation (D-dimer).
- Serum chemistry (ALT, AST, alkaline phosphatase, GGT, LDH, total bilirubin, direct bilirubin, glucose [fasting], sodium, potassium, calcium (albumin adjusted calculation), magnesium, BUN, creatinine, calculated creatinine clearance [Cockcroft-Gault equation], CPK, troponin T [high sensitivity], NT-pro-BNP, albumin, amylase, lipase, and procalcitonin).
- Immunology (CRP, ferritin, IL-1β, IL-6, IL-10, and TNFα).
- Quantitative PCR for COVID-19 viral load (*Day 8 only*).
- Serological SARS-CoV-2 testing (IgG) (*Day 31 only and at end of study visit*).
- Chest imaging (using same technique as baseline).
- Concomitant medications.
- Adverse events/TEAEs.
- Functional status using the Barthel index (Appendix 7) score.
- Clinical status, using the 11-point WHO Clinical Progression Scale (highest category that applies) (Appendix 13).
- Administration of dexamethasone base will be continued at 6 mg/day PO/IV (equivalent to 7.2 mg dexamethasone phosphate) up to a total cumulative dose of 60 mg, per physician judgement based on patient clinical condition and evolution.

7.4.2.4. Day 15

The following assessments will be performed on Day 15 (±1), in remote or on-site visit:

- Concomitant medications.
- Adverse events/TEAEs.
- Functional status, using the Barthel index (Appendix 7) score.
- Clinical status, using the 11-point WHO Clinical Progression Scale (highest category that applies) (Appendix 13).

7.4.3. End of Study

End of study visit: all subjects will undergo safety and efficacy follow-up assessments on the protocol-specified Day 31 (±3 days), earlier if patient withdraw from study or when treatment-related adverse events, treatment-emergent adverse events of special interest, and SAEs will be resolved/stabilised.

7.4.4. Study Schedule

A schedule of study assessments is provided in Appendix 5.

8. SAMPLE SIZE AND DATA ANALYSES

A general description of the statistical methods to be used to analyse safety and efficacy data is outlined below. Specific details will be provided in the statistical analysis plan (SAP).

8.1. Determination of Sample Size

This is a multicentre, open-label, controlled Phase 3 study in which adults requiring hospital admission and O_2 supplementation for management of moderate COVID-19 infection will be randomised 1:1:1 to:

- Plitidepsin 1.5 mg arm: Patients will receive plitidepsin 1.5 mg/day IV in addition to dexamethasone on Days 1 to 3 (See Section 5. Study treatments);
- Plitidepsin 2.5 mg arm: Patients will receive plitidepsin 2.5 mg/day IV in addition to dexamethasone on Days 1 to 3 (See Section 5. Study treatments);
- Control arm: Patients will receive dexamethasone on Days 1 to 3. Additionally, in accordance with local treatment guidelines, patients in this group may receive a regulatory-approved antiviral treatment (See Section 5. Study treatments).

In case of patients in both plitidepsin arms, dexamethasone in Days 1 to 3 is administered as a premedication. The study allows up to a total cumulative dose of 60 mg of dexamethasone base (calculation of the total dose will also include corticosteroids administered within 72 hours before the start of the study treatment and dexamethasone administered as premedication).

Randomisation will be stratified for 3 factors:

a) Geographical Region (Europe *versus* Rest of the World);

b) Charlson Comorbidity Index (0-1 *versus* >1) (Appendix 11 - Age-adjusted Charlson Index); and

c) Barthel Index (≥90 *versus* <90) (Appendix 7 - Barthel Index for Functional Assessment)

From treatment initiation, patients will be followed in the hospital for at least 4 days and then through Day 31 (±3 days) or resolution/stabilisation of treatment-related adverse events, treatment emergent adverse events of special interest and SAEs that occurred through Day 31.

A total sample size of 609 patients (203 in each arm) with 530 events necessary for the analysis of the primary endpoint has been calculated based on assumptions of a 1-sided type I error of 1.25% (α-0.0125) with at least 80% power (β=0.2) to detect target hazard ratio (HR) of 1.4 in the time to sustained withdrawal of supplementary oxygen, which means a decrease in the median time to the event from 8 days[48] (control arm) to 5.7 days (plitidepsin). This sample size is adjusted for the multiple comparisons of each plitidepsin arm with control group by a Bonferroni adjustment. At the final analysis, if the HR is 1.27 or greater, in favour of any plitidepsin arm (equivalent to a decrease in the median time to sustained withdrawal of supplementary oxygen of 1.7 days or greater), then it is expected that the null hypothesis (i.e., HR≤1) will be rejected.

A futility analysis for efficacy/safety will be performed when 33% of events (sustained withdrawal of supplementary oxygen) have been reached.The rho family of beta-spending functions (ρ=1.18) will be used for the calculation of the stopping boundaries for futility in order to control type II error. The stopping boundaries for the futility analysis will be calculated with the actual number of events available in the ITT population at that moment; if the number of

events at the futility analysis is 116 in a pair comparison, the beta-spending corrected p-value to reject the alternative hypothesis should be higher or equal than 0.4199 and this p-value is associated with a HR≤1.0382.

If the recommendation of the IDMC is to discontinue one of the plitidepsin dosing arms at this futility analysis following the stopping rules, the study will continue from that point on a 1:1 randomisation fashion until the completion of the remaining treatment arms (203 patients per arm and 353 events of sustained withdrawal of supplementary oxygen in total necessary to perform the final analysis). The patients still ongoing in the dropped arm would continue to be followed up as per protocol.

8.2. Analysis Populations

Analysis of the primary and other time-to-event efficacy endpoints will be performed in the ITT population. Other secondary efficacy endpoints will be based on the FAS population. Supportive analyses will be performed on the Per Protocol population. A sensitivity analysis will be performed for the primary endpoint and other time-to-event efficacy endpoints in the FAS population, Safety endpoints analyses will be based on the Safety population.

8.2.1. Intent-to-Treat (ITT) Population

All patients randomised in the trial, regardless of whether they received treatment.

8.2.2. Full Analysis Set (FAS) Population

All randomised patients who have taken at least 1 dose of study treatment (plitidepsin or control) and with at least 1 postbaseline clinical status collected. FAS population will be analysed according to their randomised treatment.

8.2.3. Per Protocol Population

A subgroup of the FAS population that includes all patients who do not have any important protocol deviations that would interfere with the collection or interpretation of the efficacy data. Important protocol deviations will be defined in the SAP. Per Protocol population will be analysed according to their randomised treatment. Supportive efficacy analyses will be performed on this population.

8.2.4. As Treated Population

All patients who received any exposure to study treatment (plitidepsin or control). As Treated population will be analysed according to the treatment they actually received.

8.3. General Considerations

Demographic and baseline disease characteristic data will be summarised descriptively.

All summary tables for quantitative parameters will display n, mean, standard deviation, median, interquartile range (25th and 75th percentiles), and range (minimum and maximum). All summary tables for qualitative parameters will display counts and percentages.

The use of imputation methods in visits of patients with missing values will be detailed in the SAP.

8.4. Study Treatment Exposure

The study treatment exposure will be assessed by study treatment (plitidepsin, dexamethasone, and any regulatory-approved antiviral treatment, such as remdesivir or favipiravir) as the duration of treatment exposure during the study. The duration of study treatment exposure will be calculated by study treatment as: (date of the last study treatment taken – date of the first study treatment taken) + 1.

8.5. Efficacy Analysis

Analysis of the primary, key secondary and other time to event endpoints will be performed in the ITT population, and other secondary efficacy endpoints will be based on the FAS population. A sensitivity analysis will be performed for the primary endpoint and other time-to-event efficacy endpoints in the FAS population. Safety endpoints analyses will be based on the Safety population. Supportive analyses will be performed on the Per Protocol population.

8.5.1. Primary Efficacy Outcome Measures

Time-to event data (time to sustained withdrawal of oxygen supplementation) will be analysed using the Kaplan Meier method and presented with 95% CIs. A Cox regression model, including the levels of the randomisation stratification factors as covariates, will be applied to compare results between each dose of plitidepsin *versus* control arm. Hazard ratios, 2-sided 95% CIs, and p values for the comparison of each dose of plitidepsin *versus* control arm will be presented. The time-to-event between each dose of plitidepsin *versus* control arm will be compared using a 2-sided stratified log-rank test. The model will be adjusted for the randomisation stratification factors, i.e., Geographical Region (Europe *versus* Rest of the World), Charlson Comorbidity Index (0-1 *versus* >1) (Appendix 11- Age-adjusted Charlson Index) and Barthel index (≥90 *versus* <90) (Appendix 7 – Barthel Index for Functional Assessment). An unstratified log-rank test and Cox regression model will also be analysed as a sensitivity analyses. For both stratified and unstratified models, the hazard ratios, 2-sided 95% CIs, and p-values for the comparison of each dose of plitidepsin *versus* control arm will be presented. Proportional hazard assumption will be checked by means of a Cox regression including treatment and its interaction with time.[49] In case of strong rejection of proportionality, then restricted mean survival estimates and weighted log-rank tests (if applicable) will also be calculated in a sensitivity analysis in addition to HR.[50] The main analysis will be adjusted for multiplicity using the Hochberg step-up procedure to preserve the type I error rate in the comparison of each dose of plitidepsin *versus* control arm. If the accrual in any of the experimental arms is stopped after the interim futility analysis, the final analysis for the primary endpoint in the remaining arms will be performed at the 1.25% level using a Bonferroni-adjusted type I error rate.

8.5.2. Key Secondary Efficacy Outcome Measures

Time-to event data will be analysed using the Kaplan-Meier method and presented with 95% CIs. A Cox-regression model, including the levels of the randomisation stratification factors as covariates, will be applied to compare results between each dose of plitidepsin *versus* control arm. Hazard ratios, 2-sided 95% CIs, and p-values for the comparison of each dose of plitidepsin *versus* control arm will be presented.

In the event that the primary endpoint is significant for both doses, the Hochberg procedure will be used to test the key secondary efficacy endpoints at the overall (2-sided) significance level of 0.05. This adjustment will not apply if the accrual in any of the experimental arms is stopped after the interim futility analysis. Details will be provided in the SAP.

8.5.3. Other Secondary Efficacy Outcome Measures

Descriptive statistics for the other secondary efficacy endpoints will be presented by treatment group and by study visit where appropriate. We will use similar methods as specified for the analyses of the primary and secondary variables.

For binary endpoints, the treatment group comparisons will be performed at Day 8 (±1 day) only, with descriptive statistics provided for each clinic visit.

Longitudinal analyses will be also performed assuming mixed-effect models including treatment effect, time effect, randomisation stratification factor and a time-treatment interaction as covariates, using a covariance structure of autoregressive order 1.

The clinical status, as assessed on the 11-point WHO Clinical Progression Scale (Appendix 13) on Day 8 (±1 day), will be summarised by treatment group. The clinical status at Day 8 (±1 day) between each dose of plitidepsin *versus* control arm will be compared using a proportional odds model. The model will be adjusted for the randomisation stratification factors. The odds ratios, 2-sided 95% Cis, and p-values for the comparison of each dose of plitidepsin *versus* control arm will be presented.

8.6. Subgroup Analyses

The primary and key secondary efficacy endpoints will be analysed by the following subgroups:

- Geographical Region (Europe *versus* Rest of the World).
- Charlson Comorbidity Index: 0-1 *versus* >1 (Appendix 11 – Age-adjusted Charlson Index).
- Barthel index (≥90 *versus* <90) (Appendix 7 – Barthel Index for Functional Assessment).

Additionally, the primary efficacy endpoint will be analysed by the following subgroups:

- Sex: female or male.
- Age at study entry: 18 to 39, 40 to 64, ≥65 years.
- Race: Asian, black, white, or other.

- Ethnic group: Hispanic or Latino or Not Hispanic or Latino.
- Site location: analyses will be performed to assess that no single study site provided an unusually large fraction of the patients and that no single investigator or site was disproportionately responsible for the favourable effect seen.
- User of antiviral therapies or immunomodulatory drugs.
- Previous COVID-19 vaccination status (complete/incomplete/no).
- Anti-SARS-CoV-2 IgG Day 1 (non-positive/positive).
- Pre-randomisation dexamethasone (yes/no).
- Total duration of corticoid therapy (±10 days).
- Total cumulative dose of corticoid therapy (≤ 60 or >60 mg dexamethasone or equivalent) (See Appendix 15 – Glucocorticoids equivalent anti-inflammatory doses).
- Days of symptoms (≤5 days, 5-10 days, > 10 days).
- Administration of approved antivirals if the patient is randomised to the control arm (remdesivir/favipiravir/no).
- Other exploratory subgroup analyses may be prespecified in the SAP based on prognostic factors of medical interest (e.g., baseline measures of the disease status or laboratory parameters such as, LDH, CRP, D-dimer, ferritin, etc.).
- SARS-CoV-2 variant analysis (United Kingdom α, South Africa β, Brazil γ, India δ, California ε, Peru λ, and New York ι) and potential new variants when emerging in the selected countries.[51]
- Patients included before and after amendment (protocol v.6 *versus* v.7).

8.7. Safety Analysis

Safety analyses will be based on the As Treated Population. All safety parameters will be summarised and also listed by patient.

Treatment-emergent Adverse Events: The verbatim terms used in the eCRF by investigators to identify TEAEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and will be graded according to NCI CTCAE version 5.0. All reported AEs that occur or worsen on or after the first dose of study drug (Day 1) up through Day 31 (±3 days) (last protocol-specified follow-up visit) will be included in the analysis. Treatment-emergent AEs will be summarised by MedDRA system organ class and preferred term. For each TEAE, the percentage of subjects who experience at least 1 occurrence of the TEAE will be summarised overall and by treatment group. The SAEs, AEs of special interest, and deaths will be listed. All TEAEs resulting in discontinuation of study treatment, dose modification, dose interruption, or a delay in treatment with the study drug will be listed and summarised by system organ class/preferred term.

Clinical Laboratory Tests: Laboratory data will be summarised by type of laboratory test. The worst toxicity grade will be tabulated. Parameters with predefined NCI CTCAE version 5.0 toxicity grades will be summarised. Change from baseline to the worst TEAE grade experienced by the subject during the study will be provided as shift tables.

Electrocardiograms: The effects of treatment on QTcF interval, including events of QTcF >500 msec and >60 msec from baseline, will be evaluated by means of descriptive statistics and frequency tabulations.

Subgroup analysis of TEAEs and clinically relevant laboratory tests will be performed based on prognostic factors of medical interest (e.g., sex, age, administration of remdesivir/favipiravir in the control arm, etc.).

8.8. Pharmacokinetic Analysis

Only applicable for Appendix 8 (Protocol for QTc Substudy).

8.9. Futility Analysis

A futility analysis for efficacy/safety will be performed when 33% of events (sustained withdrawal of supplementary oxygen) have been reached. The rho family of beta-spending functions (with ρ=1.18) will be used for the calculation of the stopping boundaries for futility in order to control type II error. The stopping boundaries for the futility analysiswill be calculated with the actual number of events available in the ITTpopulation at that moment; if the number of events at the futility analysis is 116 in a pair comparison patients, the beta-spending p-value to reject the alternative hypothesis should be higher or equal than 0.4993 and this p-value is associated with a HR≤1.0382.

If the recommendation of the IDMC is to discontinue one of the plitidepsin dosing arms at this futility analysis following the stopping rules, the study will continue from that point on a 1:1 randomisation fashion until the completion of the remaining treatment arms (203 patients per arm and 353 events of sustained withdrawal of supplementary oxygen in total necessary to perform the final analysis). The patients still ongoing in the dropped arm would continue to be followed up as per protocol and a Bonferroni-adjusted type I error rate of 1.25% will be kept for the comparison of the primary endpoint between the remaining plitidepsin arm and the control arm.

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10. APPENDICES

Appendix 1 – Adverse Event Definitions

In emergency situations, the investigator should immediately get in touch with the sponsor's pharmacovigilance contact at the telephone number or email address given on the title page of this protocol.

Patients will be evaluable for safety if they have received at least part of 1 plitidepsin infusion/control arm. Adverse events (AEs) will be graded according to the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0. [*http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf*]

Treatment compliance, i.e., dose delays, dose reductions and reasons for treatment discontinuation, will be monitored throughout the study.

The safety profile of patients will be monitored throughout the treatment and up to 28 days (±3 days) after the last treatment infusion, or until the date of death, whichever occurs first.

Any treatment-emergent AEs (TEAE; the term TEAE covers any unfavourable or unintended sign, symptom, syndrome, or illness, including laboratory abnormalities that develop or worsens during the period of observation in the clinical study) will be followed until recovery to at least Grade 1 or stabilisation of symptoms or death, whichever occurs first. After treatment discontinuation, patients will be followed until resolution or stabilisation of all toxicities, if any. Patients having any treatment-related Grade ≥3 AEs should have relevant tests re-assessed at least every 72 hours until recovery to at least Grade 2.

Adverse Event Definitions

Adverse Event

An AE is any untoward medical occurrence in a patient or clinical investigation patient administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign, (e.g., an abnormal laboratory finding), or a disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Illnesses with onset during the study or exacerbations of pre-existing illnesses, including but not limited to clinically significant changes in physical examination findings and abnormal objective tests/procedures findings (e.g., X-ray, electrocardiogram) should be recorded. The criteria for determining whether an abnormal objective test finding should be reported as an AE are as follows:

- The test result is associated with clinically significant symptoms, and/or
- The test result leads to a change in the study dosing or discontinuation from the clinical trial, significant additional concomitant drug treatment or other therapy, and/or

- The test result leads to any of the outcomes included in the definition of a serious AE (SAE; see definition below), and/or
- The test result is considered to be clinically relevant by the investigator.

Serious Adverse Event

A SAE is defined as any adverse experience occurring at any dose that:

- results in death (is fatal)
- is life-threatening
- requires or prolongs inpatient hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect, or
- is medically significant.
- any suspected transmission via a medicinal product of an infectious agent.

Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations, such as an important medical event that may not be immediately life-threatening or result in hospitalisation but may jeopardise the patient or may require intervention to prevent one of the outcomes listed in the above definition.

Death

Death is the outcome of an SAE and should not be used as the SAE term itself. The cause of death should be recorded as the SAE term instead. When available, the autopsy report will be provided to the sponsor.

Life-threatening Event

A life-threatening event is defined as any event in which the subject is at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

Hospitalisation/Prolongation of Hospitalisation

Any event requiring hospitalisation (or prolongation of hospitalisation) that occurs or worsens during the course of a patient's participation in a clinical study must be reported as an SAE unless exempted from SAE reporting. Prolongation of hospitalisation is defined as any extension of an inpatient hospitalisation beyond the stay anticipated/required for the initial admission, as determined by the investigator or treating physician.

Hospitalisations that do not meet criteria for SAE reporting are:

- Reasons described in the protocol (e.g., investigational medicinal product [IMP] administration, protocol-required investigations). However, events requiring

hospitalisation or prolongation of hospitalisation as a result of a complication of therapy administration or clinical study procedure will be reported as an SAE.

- Hospitalisation or prolonged hospitalisation for technical, practical or social reasons, in the absence of an AE.
- Pre-planned hospitalisations: Any pre-planned surgery or procedure must be documented in the source documentation. Only if the pre-planned surgery needs to be performed earlier due to a worsening of the condition should this event (worsened condition) be reported as an SAE.

Other situations that MUST NOT be considered as hospitalisations are:

- An emergency visit due to an accident where the patient is treated and discharged.
- When the patient is held 24 hours for observation and is finally not admitted.
- Planned treatments at sites not associated to a hospital and generally considered as minor surgical procedures (i.e., laser eye surgery, arthroscopy, etc.).

Unexpected/Unlisted Adverse Event

An AE is considered unexpected/unlisted when the nature or severity of which is not consistent with the applicable reference safety information (RSI). The sponsor will use as the RSI for the evaluation of listedness/expectedness the RSI of the most updated IB for plitidepsin.

Adverse Reaction

All untoward and unintended response to an IMP related to any dose administered. This definition also covers medication errors and uses outside what is foreseen in the protocol, including overdose, misuse and abuse of the product.

Adverse Events Related to the Study Drug

An AE is considered related to a study drug/IMP if the investigator's and/or the sponsor's assessment of causal relationship to the IMP(s) is "Y (yes)". The investigator will assess the causal relationship of the IMP(s) to the SAE. The sponsor will consider related to the study drug(s)/IMP(s) those events for which the investigator and/or the sponsor assesses the causal relationship with the IMP(s) as "Uk (unknown)" when it cannot rule out a role of the IMP(s) in the event.

Expedited Reporting

The sponsor is responsible for appropriate expedited reporting to the competent authorities, the investigators, and the IEC/IRB according to current legislation.

Assessment of Causal Relationship to the Study Drug

The investigator must provide an assessment of causality for the IMP according to the following criteria:

- Y (yes): there is a reasonable possibility that the IMP(s) caused the AE/SAE.
- N (no): there is NO a reasonable possibility that the IMP(s) caused the AE/SAE and other causes are more probable.
- UK (Unknown): only to be used in special situations where the investigator has insufficient information (i.e., the patient was not seen at his/her centre). If none of the above can be used.

Adverse Event Reporting Procedures

Reporting of Adverse Events

The sponsor will collect all AEs until 28 days (±3 days) after administration of the last dose of any of the study arms (plitidepsin and control arm) or until the date of death, whichever occurs first. All AEs suspected to be related to the study drug(s)/IMP(s) must be followed-up after the time of therapy discontinuation until the event or its sequelae resolve or stabilise at an acceptable level to the investigator and the sponsor.

All AEs, including medication errors and uses outside what is foreseen in the protocol, must be recorded in English using medical terminology in the source document and the eCRF. Whenever possible, the investigator will record the main diagnosis instead of the signs and symptoms normally included in the diagnoses.

Investigators must assess the severity (Grade) of the event following the NCI-CTCAE version 5.0 and assign a relationship to each study drug(s)/IMP(s); and pursue and obtain information adequate both to determine the outcome and to assess whether it meets the criteria for classification as an SAE requiring immediate notification to the sponsor or its designated representative. The investigator must provide any relevant information as requested by the sponsor in addition to that on the eCRF.

Abnormal laboratory tests occurring during the study must only be recorded in the AE section of the eCRF if the disorder:

- Is associated with clinically significant symptoms, and/or
- Leads to a change in study dosing (omission, delay or reduction) or discontinuation from the study, significant additional concomitant drug treatment or other therapy, and/or
- Leads to any of the outcomes included in the definition of an SAE.

Otherwise, laboratory results should be reported in the corresponding section of the eCRF (e.g., biochemistry, haematology).

The “Adverse event form” will be used only for events that occur after the first drug infusion or any event related to a study procedure within the study period (according to International Conference on Harmonisation [ICH] guidelines); and to report ‘ongoing’ baseline conditions in case of any significant change (improvement or worsening) during the study.

Reporting of Serious Adverse Events

The sponsor will collect all SAEs from the time of signing of the ICF and until 28 days (±3 days) after administration of the last dose of any of the study arms (plitidepsin and control arm) or until death, whichever occurs first. Beyond this period of time, only those SAEs suspected of being related to the IMPs will be collected. Nonetheless, the sponsor must evaluate any safety information related to the clinical study that is spontaneously reported by an investigator beyond the time frame specified in the protocol.

All SAEs (as defined above) that have occurred after patient registration, regardless of relationship to the study drug(s)/IMP(s), must be reported immediately, and always within 24 hours to the Pharma Mar S.A. Pharmacovigilance Department electronically by completing the applicable eCRF section. Only in situations of electronic system failure or pregnancy exposure during the clinical trial, can SAEs be reported using paper on a "SAE form by fax (+34 91 846 6004) or email (AEincoming@pharmamar.com). Outside of office hours (Greenwich Meridian Time [GMT]), assistance on SAE reporting can be obtained by calling the Pharmacovigilance Department at +34 681 263 592. In case of electronic system failure or pregnancy exposure during the clinical trial, SAEs initially reported by alternative methods (not electronically), must be followed by completed electronic SAE reporting on an eCRF from the investigational staff within 1 working day.

The SAEs occurring during the screening phase (from ICF signature to registration) and after off-study will be reported using a paper "SAE form" that must be forwarded as mentioned above always within 24 hours to the Pharma Mar S.A. Pharmacovigilance Department by fax or email.

All SAEs suspected to be related to the study drug must be followed until the event or its sequelae resolves or stabilises to at least Grade 1, or to an acceptable level according to the investigator and the sponsor or his/her designated representative.

Reporting of Pregnancy Cases Occurring During the Clinical Study

National regulations require that the sponsor collect information on pregnancies occurring during clinical trials, in which exposure to the study drug at any time during pregnancy, via either maternal or paternal exposure, is suspected.

Therefore, pregnancy and suspected pregnancy (including a positive pregnancy test regardless of age or disease state) of a female patient or the female partner of a male patient occurring while the patient is on study drug, or within 31 days of the patient's discontinuation visit, are considered immediately reportable events.

The investigator will report the following events immediately and always within 24 hours from first knowledge:

- Any occurrence of a pregnancy where any kind of exposure to the study drug is suspected.
- Possible exposure of a pregnant female.

- All reports of elevated/questionable or indeterminate beta human chorionic gonadotropins.

Immediately after detecting a case of suspected pregnancy in a female clinical trial patient, the decision on her continued participation in the clinical trial will be jointly taken by the trial patient, the investigator and the sponsor with the patient's best interest in mind. A decision to continue the pregnancy will require immediate withdrawal from the trial.

Any pregnancy, suspected pregnancy, or positive pregnancy test must be reported to the Pharmacovigilance Department at Pharma Mar immediately by facsimile or email using the pregnancy report form.

The investigator will follow the pregnancy until completion/termination, and must notify the outcome of the pregnancy to the Pharmacovigilance Department at Pharma Mar within 24 hours of first knowledge as a follow-up to the initial pregnancy report.

For any event during the pregnancy which meets a seriousness criterion (including foetal or neonatal death or congenital anomaly), the investigator will also follow the procedures for reporting SAEs (complete and send the SAE form to Pharmacovigilance Department at Pharma Mar by facsimile (+34 91 846 6004) or by email (AEincoming@pharmamar.com) within 24 hours of the investigator's knowledge of the event).

All neonatal deaths that occur within 30 days of birth should be reported, regardless of causality, as SAEs. In addition, any infant death at any time thereafter that the investigator suspects as related to the in-utero exposure to the study drug should also be reported to the Pharmacovigilance Department at Pharma Mar by facsimile within 24 hours of the investigators' knowledge of the event.

Adverse Events Monitoring

Safety review will be performed at Pharma Mar S.A. once the SAE forms have been received and the eCRFs have been completed by the investigator.

Periodic safety review of clinical data will be performed; however, no formal Data Safety Monitoring Board has been appointed for this trial. The AEs will be monitored by the investigators and by the study team at Pharma Mar S.A. The personnel in charge of this process are defined in the section "Study Contacts" of this protocol. In general, a clinical virologist, together with a member of the Pharma Mar S.A. Pharmacovigilance Department will review the safety data of this trial on an ongoing basis.

- SAEs will be collected, assessed and reported as per the applicable regulations by the Pharmacovigilance Department.

- Nonserious AEs will be checked for accuracy against the eCRF during monitoring visits by the monitor.

Appendix 2 – Clinical Laboratory Evaluations

The following clinical laboratory analytes will be assessed:

Serum chemistry	**Haematology**
Alkaline phosphatase	Haematocrit
Alanine aminotransferase	Haemoglobin
Aspartate aminotransferase	Platelet count
Blood urea nitrogen	Red blood cell count
Creatinine, calculated creatinine clearance (Cockcroft-Gault equation)	White blood cell count
Gamma-glutamyl transferase	Basophils
Sodium, potassium, calcium (albumin adjusted calculation), magnesium	Eosinophils
Glucose (fasting)	Lymphocytes
Lactate dehydrogenase	Monocytes
Creatine phosphokinase	Neutrophils
Troponin (I or T at screening per local laboratory and T at Day 1 onward by central laboratory)	Bands/immature granulocyte
NT-pro-BNP	**For females only:**
Amylase	Serum or urine pregnancy test
Total bilirubin	**Immunology:**
Direct bilirubin	C-reactive protein
Lipase	Ferritin
Procalcitonin	Interleukin-1β
Albumin	Interleukin-6
Coagulation:	Interleukin-10
D-dimer	Tumour necrosis factor alpha
Serology	
Immunoglobulin G	

Abbreviations: NT-pro-BNP = N-terminal-pro-B-type natriuretic peptide

Appendix 3 – Regulatory, Ethical, and Study Oversight Considerations

Good Clinical Practice

This clinical study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki compliance with the protocol, GCP as defined the ICH Guidance E6: *Good Clinical Practice: Consolidated Guidance* and applicable federal and local regulatory requirements.

Delegation of Investigator Duties

The investigator should ensure that all persons assisting with the trial are adequately qualified, informed about the protocol, any amendments to the protocol, the study treatments, and their trial-related duties and functions. The investigator should maintain a list of subinvestigators and other appropriately qualified people to whom he/she has delegated significant trial-related duties.

Patient Information and Informed Consent

Before enrolling in the study or performing any study-specific procedures (including screening and baseline tests), patients must give written informed consent to participate in the study. Each consent must be confirmed by the patient's dated signature and the name and dated signature of the principal investigator or subinvestigator conducting the informed consent discussions. A copy of the informed consent will be given to the patient. The original consent documents will be retained in the investigator study file.

The informed consent and assent documents will contain all the information required by the ICH Guidance E6: *Good Clinical Practice: Consolidated Guidance* and any additional elements required by local regulations. In addition to the document, the investigator should provide oral information and answer questions from the subject. Subjects should be given adequate time for thought and to ask questions.

Confidentiality

All data and records generated during this study will be kept confidential in accordance with Institutional policies, GDPR and Regulation (EU) 536/2014, on subject privacy and that the investigator and other site personnel will not use such data and records for any purpose other than conducting the study.

Patient names will not be provided to the sponsor. Only the unique patient identification number will be recorded in the eCRF. If the patient's name appears on any document (e.g., laboratory report), the name must be eliminated/redacted on the copy of the document supplied to the sponsor. Study findings stored on a computer will be kept in accordance with local data protection laws. Patients will be informed that representatives of the sponsor, independent ethics committee (IEC)/Institutional Review Board (IRB), or regulatory authorities may review their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

The investigator will maintain a patient identification list (unique patient identification number with the corresponding patient name) to enable records to be identified.

Approval of the Clinical Study Protocol and Amendments

Before the start of the study, the clinical study protocol, the informed consent document and informed assent document, and any other appropriate documents will be submitted to the IEC/IRB and other applicable national competent authorities. As this study is being conducted globally, approval for these study documents must be received from the IEC/IRB and applicable national competent authorities before the study is initiated at a given investigational site.

The IEC/IRB and applicable national competent authorities must be informed of all administrative changes and any important finding that could modify the risk of exposed subjects. They must also be informed or their authorisation obtained for all subsequent amended protocols, in accordance with local legal requirements. The investigator must keep a written record of all communications with the IEC/IRB and applicable national competent authorities.

Ongoing Information for IEC/IRB and Health Authorities (as per Local Regulations)

The sponsor must submit the following to all investigators, the IEC/IRB, and Health Authorities:

- Information on serious or unexpected adverse events from any investigational site, as soon as possible
- Expedited safety reports, in accordance with local and national regulations
- Periodic reports on study progress (at least annually)

Record Retention

Study documents should be retained by the investigator as long as required by local regulations. Beyond this period, the investigator must still obtain approval in writing from the sponsor before destruction of any records.

The documents to be retained include:

- Original signed informed consent and informed assent documents for all subjects
- Patient identification code list, screening log, and enrolment log
- Record of all communications between the investigator and the IEC/IRB
- List of subinvestigators and other appropriately qualified persons to whom the investigator has delegated significant trial-related duties, together with their roles in the study and their signatures
- Copies of the eCRFs and documentation of corrections on Data Correction Forms for all subjects
- Investigational product accountability records
- Record of any body fluids or tissue samples retained
- All other source documents (patient medical records, hospital records, laboratory records, etc.)

- All other documents as listed in Section 8 (Essential Documents for the Conduct of a Clinical Trial) of the ICH Guidance E6: *Good Clinical Practice: Consolidated Guidance*.

Liability and Insurance

Liability and insurance provisions for this study are given in separate agreements.

The sponsor has taken out an insurance policy covering their civil responsibility.

Financial Disclosure

Before the start of the study, the investigator and each subinvestigator will disclose to the sponsor any proprietary or financial interests he or she may hold in the investigational products or the sponsor company as outlined in the financial disclosure form provided by the sponsor. The investigator and each subinvestigator also agree to update this information in case of significant changes during the study or within 1 year of study completion. The investigator and each subinvestigator also agree that, where required by law or regulation, the sponsor may submit this financial information to domestic or foreign regulatory authorities in applications for marketing authorisations.

Study Monitoring and Auditing

Monitoring and auditing procedures developed or endorsed by the sponsor will be followed to comply with GCP guidelines. Direct access to the on-site study documentation and medical records must be ensured.

Monitoring and Source Document Verification

Monitoring will be done by personal visits from representatives of the sponsor (clinical research assistant and medical monitor) who will check the eCRFs for completeness and clarity, and crosscheck them with source documents. In addition to the monitoring visits, frequent communications (letter, telephone, email and fax) by the study monitors will ensure that the investigation is conducted according to the protocol design and regulatory requirements. Source document verification may be performed remotely, if required.

Study close-out will be performed by the study monitor upon closure of the study.

On-site Audits/Inspections

An external auditor appointed by the sponsor or the IEC/IRB as well as inspectors appointed by domestic and foreign regulatory authorities may request access to source documents, eCRFs, and other study documents for on-site audits or inspections. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities. Medical records and other study documents may be copied during audit or inspection provided that patient names are obliterated on the copies to ensure confidentiality. Audits/inspections may be performed remotely, if required.

Documentation and Use of Study Findings

Documentation of Study Findings

All protocol-required information collected during the study must be entered by the investigator, or designated representative, in the eCRF. Details of eCRF completion and correction will be explained to the investigator. If the investigator authorises other persons to make entries in the eCRF, the names, positions, signatures, and initials of these persons must be supplied to the sponsor.

The investigator, or designated representative, should complete the eCRF as soon as possible after data are collected, preferably on the same day the patient is seen for an examination, treatment, or any other study procedure and at the latest before the next monitoring visit. An explanation should be given for all missing data.

A source data location list will be prepared before study starts. This list will be filed in both the trial master file and the investigator study file and updated as necessary.

The completed eCRFs must be reviewed and signed by the investigator named in the clinical study protocol or by a designated co-investigator.

Confidentiality/Use of Study Findings

All information concerning the product and any matter concerning the operation of the sponsor, such as clinical indications for the drug, its formula, methods of manufacture and other scientific data relating to it, that have been provided by the sponsor and are unpublished, are confidential and must remain the sole property of the sponsor. The investigator will agree to use the information only for the purposes of carrying out the study and for no other purpose unless prior written permission from the sponsor is obtained.

The sponsor has full ownership of the original eCRFs completed as part of this study.

By signing the clinical study protocol, the investigator agrees that the results of the study may be used for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. The authorities will be notified of the investigator's name, address, qualifications, and extent of involvement.

The sponsor will ensure that a clinical study report on the study is prepared.

Irrespective of the outcome of a clinical trial, within one year from the end of a clinical trial in all Member States concerned, the sponsor will submit to the EU/national database a summary of the results of the clinical trial.

All materials, documents, and information provided by the sponsor to the investigator, and all materials, documents and information prepared or developed in the course of the study to be performed under this protocol, shall be the sole and exclusive property of the sponsor. Subject to obligations of confidentiality, the investigator reserves the right to publish only the results of work performed pursuant to this protocol provided; however, that the investigator provides an

authorised representative of the sponsor with a copy of any proposed publication for review and comment at least 45 days in advance of its submission for publication. If requested, the investigator will withhold publication an additional 90 days to allow for filing a patent application or taking such other measures the sponsor deems appropriate to establish and preserve its proprietary rights.

It is agreed that, consistent with scientific standards, publication of the results of the study shall be made only as part of a publication of the results obtained by all sites performing the protocol.

Appendix 4 – Inhibitors and Inducers of CYP3A4

Strong inhibitors and inducers of cytochrome P450 3A4 (CYP3A4) are prohibited, as they may affect the systemic exposure of plitidepsin. Moderate and weak inhibitors and inducers of CYP3A4 may be used with caution in combination with plitidepsin.

Classification of *in vivo* Inhibitors of CYP3A4 Enzyme

Strong Inhibitors (2) **≥5-fold Increase in AUC or >80% Decrease in CL**	**Moderate Inhibitors (3)** **≥2 but <5-fold Increase in AUC or 50% to 80% Decrease in CL**	**Weak Inhibitors (4)** **≥1.25 but <2-fold Increase in AUC or 20% to 50% Decrease in CL**
boceprevir, clarithromycin, cobicistat, danoprevir and ritonavir, elvitegravir and ritonavir, grapefruit juice, idelalisib, indinavir and ritonavir, itraconazole, ketoconazole, lopinavir and ritonavir, nefazodone, nelfinavir, paritaprevir and ritonavir and (ombitasvir and/or dasabuvir), posaconazole, ritonavir, saquinavir and ritonavir, telaprevir, tipranavir and ritonavir, telithromycin, troleandomycin, voriconazole	aprepitant, ciprofloxacin, conivaptan, crizotinib, cyclosporine, diltiazem, dronedarone, erythromycin, fluconazole, fluvoxamine, imatinib, tofisopam, verapamil	chlorzoxazone, cilostazol, cimetidine, clotrimazole, fosaprepitant, istradefylline, ivacaftor, lomitapide, ranitidine, ranolazine, ticagrelor

Notes: This is not an exhaustive list. For an updated list, see the following link: Drug Development and Drug Interactions | Table of Substrates, Inhibitors and Inducers | FDA #Table3-2

- A strong inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a substrate for that CYP by equal or more than 5-fold.
- A moderate inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a sensitive substrate for that CYP by less than 5-fold but equal to or more than 2-fold.
- A weak inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a sensitive substrate for that CYP by less than 2-fold but equal to or more than 5-fold.

Classification of *in vivo* Inducers of CYP3A4 Enzymes

Strong Inducers **≥80% Decrease in AUC**	**Moderate Inducers** **≥50% to <80% Decrease in AUC**	**Weak Inducers** **≥20% to <50% Decrease in AUC**
apalutamide, carbamazepine, enzalutamide, mitotane, phenytoin, rifampicin, St. John's wort	bosentan, efavirenz, etravirine, phenobarbital, primidone	armodafinil, modafinil, rufinamide

Notes: Please note the following: This is not an exhaustive list. For an updated list, see the following link: https://www.fda.gov/drugs/drug-interactions-labelling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers#table3-3.

Appendix 5 – Schedule of Assessments

Table 5: Schedule of Assessments

Procedure	Screening Day 0-1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8 (±1 day)	Day 9	Day 10	Day 15[22] (±1 day)	Day 31[23] (±3 days)
Informed consent[1]	X												
Selection criteria[2]	X												
IWRS registration	X	X											X
Medical history	X												
Vital signs[3]	X	X	X	X	X	X	X	X	X				X
Demographics	X												
Charlson Comorbidity Index	X												
Physical examination	X*	X	X	X	X				X				X
Electrocardiogram[4]	X	X		X									X
Haematology[5]	X	X	X	X	X				X				X
Serum chemistry[6]	X	X	X	X	X				X				X
Coagulation[7]		X	X	X	X				X				X
Serological test (IgG)		X											X
Immunology[8]		X	X	X	X				X				X
COVID-19 screen[9]	X												
COVID-19 viral load[10]		X							X				
Chest imaging[11]		X							X				X
Pregnancy test[12]	X												
Clinical status[13]	X	X	X	X	X	X	X	X	X			X	X
Functional status[14]	X								X			X	X
Adverse events/TEAEs[15]	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications[16]	X	X	X	X	X	X	X	X	X	X	X	X	X
Dexamethasone treatment[17]		X	X	X	X	X	X	X	X	X	X		
Plitidepsin treatment[18]		X	X	X									
Remdesivir treatment[19]		X	X	X	X	X							
Favipiravir treatment[20]		X	X	X	X	X							
Premedication[21]		X	X	X	X	X							

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; BID = twice daily; BUN = blood urea nitrogen; COVID-19 = coronavirus disease 2019; CPK = creatine phosphokinase; CRP = C-reactive protein; ECG = electrocardiogram; GGT = gamma-glutamyl transferase; Ig = immunoglobulin; IL = interleukin; IV = intravenous; IWRS = interactive web response systems; LDH = lactate dehydrogenase; NIH = National Institute of Health; NT-pro-BNP = N-terminal pro B-type natriuretic

peptide; PCR = polymerase chain reaction; PO = orally; qPCR = quantitative polymerase chain reaction; QTcF = QT interval corrected using Fridericia's formula; TEAE = treatment-emergent adverse event; TNFα = tumour necrosis factor alpha.

*Body weight and height should be measured at screening.

1 **Informed consent:** Must be obtained before the patient undergoes any study-specific procedure

2 **Selection criteria**: Confirm that subject meets all inclusion criteria and none of the exclusion criteria

3 **Vital signs**: Temperature, sitting blood pressure, heart rate, respiratory rate, PaO_2 (obtained either by arterial blood gas analyses or from saturation of oxygen (SpO_2) by pulse oximetry), and its respective FiO_2 (Appendix 12 - Imputation of FiO_2 from oxygen flow values. High-flow oxygen therapy) to be measured at screening, on Days 1 to 3 just before start and end of each plitidepsin infusion (plitidepsin groups)/once daily on Days 1 to 3 (control group), further once daily while the patient is hospitalised, and on Days 8 and 31 (±3 days). To estimate the PaO_2/FiO_2 ratio, the required equation would be used for imputing PaO_2 from SpO2 (Appendix 9 – Imputation of PaO_2 from SpO_2), and the information on the oxygen delivery devices and oxygen flow prescribed would be utilised for imputation of FiO_2.

4 **ECG**: QTcF prolongation to be assessed at screening and on Days 1, 3 (postinfusion), and 31 (±3 days), and any QTcF values >500 msec will be flagged as increasing the likelihood of the treatment being proarrhythmic (per ICH Guidance E14).

5 **Haematology**: RBC, haemoglobin, haematocrit, WBC with differential, platelet count. Testing to be performed by the local laboratory for screening and by central laboratory for on-study tests (tests performed within 24 hours of screening do not have to be repeated)

6 **Serum chemistry**: *Prestudy screening assessments:* ALT, AST, total bilirubin, sodium, potassium, calcium (albumin adjusted), magnesium, creatinine, calculated creatinine clearance (Cockcroft-Gault equation), CPK, and troponin (I or T according to local practice). *On study assessments:* ALT, AST, alkaline phosphatase, GGT, LDH, total bilirubin, direct bilirubin, glucose (fasting), sodium, potassium, calcium (albumin adjusted calculation), magnesium, blood urea nitrogen, creatinine, calculated creatinine clearance (Cockcroft-Gault equation), CPK, albumin, amylase, lipase, procalcitonin, troponin T (high sensitivity), and NT-pro-BNP. Note that testing to be performed by the local laboratory for screening and by central laboratory for on-study tests; tests performed within 24 hours of screening do not have to be repeated. All serum chemistry parameters will be measured at Day 1 prior to initiation of study treatment, once daily during hospitalisation, and at follow-up visits on Days 8 (±1 day) and 31 (±3 days); except, troponin T (high sensitivity), and NT-pro-BNP that will be measured on Day 1, Days 8 (±1 day) and 31 (±3 days).

7 **Coagulation**: D-dimer

8 **Immunology:** Proinflamatory biomarkers **(**CRP, ferritin, IL-1β, IL-6, IL-10, TNFα) in each study arm from baseline (Day 1 prior to start of the study treatment) to Day 4 and Days 8 (±1 day), 15 (±1 day) and 31 (±3 days).

9 **COVID-19 screen**: Qualitative PCR, antigen test to be performed by local laboratory, or any other validated method approved by the local health authority, from appropriate biological samples collected no more than 72 hours before study treatment on Day 1 for screening test to confirm COVID-19. In case more than one is available, the more recent one will be selected.

10 **COVID-19 viral load**: Quantitative real-time reverse transcription polymerase-chain-reaction test (qRT-PCR) test to be performed by central laboratory from oro-nasopharyngeal exudate on Day 1 prior to initiating treatment and on Day 8 (±1 day).

11 **Chest imaging:** CT scan or x-ray will be done on Days 1, 8 (±1 day) and 31 (±3 days), Chest imaging performed within 48 hours of Day 1 is accepted and does not have to be repeated

12 **Pregnancy test**: Serum or urine pregnancy test for females of child-bearing potential, to be performed by local laboratory for screening

13 **Clinical Status**: Assessed using the 11-point WHO Clinical Progression Scale (Appendix 13) will be performed at screening, daily from Day 1 while hospitalised and on Days 8 (±1 day), 15 (±1 day) and 31 (±3 days), where 0= uninfected, no viral RNA detected; 1 = asymptomatic, viral RNA detected; 2 = symptomatic, independent; 3 = symptomatic, assistance needed; 4 = hospitalised, no oxygen therapy (if hospitalised for isolation only, record status as for ambulatory patient); 5 = hospitalised, oxygen by mask or nasal prongs; 6 = hospitalised, oxygen by NIV or high flow; 7 = intubation and mechanical ventilation, $pO_2/FIO_2 \geq 150$ or $SpO_2/FIO_2 \geq 200$; 8 = mechanical ventilation, $pO_2/FIO_2 < 150$ ($SpO_2/FIO_2 < 200$) or vasopressors; 9 = mechanical ventilation, $pO_2/FIO_2 < 150$ and vasopressors, dialysis or ECMO; 10 = dead.

14 **Functional Status**: Assessed by the Barthel index score (Appendix 7); the pre-baseline (i.e., > 1 month prior to screening) functional status should be recorded, in addition to Day 1 prior to initiating study treatment, at hospital discharge, and on Days 8 (±1 day), 15 (±1 day) and 31 (±3 days).

15 **Treatment-emergent adverse events (TEAEs)**: TEAEs should be documented and recorded at each visit (i.e., from initiation of study treatment on Day 1 through at least Day 31), grading severity assessed using National Cancer Institute (NCI) Common Terminology for Adverse Events (CTCAE) v5.0, and relationship to study treatment assessed by investigator judgement

16 **Concomitant medications**: Concomitant medications and treatments will be recorded for all patients from 28 days before the start of study treatment and through study Day 31 (±3 days). All COVID-19 vaccinations should be recorded, regardless of when they were given, until the end of study.

17 **Dexamethasone treatment**: On all study arms, patients will receive dexamethasone phosphate 8 mg/day IV (equivalent to 6.6 mg dexamethasone base) on Days 1 to 3 (administered as a premedication in plitidepsin arms), following by dexamethasone phosphate 7.2 mg (equivalent to 6 mg/day dexamethasone base) PO/IV from Day 4 and up to a total cumulative dose of 60 mg of dexamethasone base (as per physician judgement according to patient clinical condition and evolution). The study allows up to a total cumulative dose of 60 mg of dexamethasone base (calculation of the total dose will also include corticosteroids administered within 72 hours before the start of the study treatment and dexamethasone administered as premedication).

18 **Plitidepsin treatment**: Plitidepsin 1.5 or 2.5 mg intravenous (IV) infusion for 60 minutes will be administered on Days 1 to 3. A 30 (±5) minute window is allowed for the plitidepsin administration between each day, and plitidepsin should not be given earlier than 23.5 hours on Days 2 and 3 of previous day plitidepsin administration. No other antiviral agents to be administered during Days 1 to 3.

19 **Remdesivir treatment**: Consistent with local treatment guidelines, patients randomised to the control arm may receive remdesivir 200 mg IV on Day 1 followed by 100 mg/day IV on Days 2 to 5.

20 **Favipiravir treatment**: Consistent with local treatment guidelines, patients randomised to the control arm may receive favipiravir 1600 mg BID PO on Day 1, followed by 600 mg BID PO daily for 2 to 5 days.

21 **Premedication** (before plitidepsin infusion start): For prevention of plitidepsin-related infusion reactions, administration of the following premedications should be ideally completed 20 to 30 minutes before initiating the plitidepsin infusion and, exceptionally, up to 2 h before plitidepsin infusion start:

   a. Palonosetron 0.25 mg IV (tropisetron 5 mg IV could be considered if palonosetron is not available)
   b. Diphenhydramine hydrochloride 25 mg IV (or equivalent, such as dexchlorpheniramine maleate 5 mg)
   c. Ranitidine 50 mg IV (or equivalent, such as famotidine 20 mg IV)
   d. Dexamethasone phosphate 8 mg IV (equivalent to 6.6 mg of dexamethasone base)

   Additionally, patients treated with plitidepsin must receive tropisetron 5 mg PO/IV on Days 4 and 5 if tropisetron 5 mg IV was administered on Days 1, 2, and 3. These premedications and postmedications should be recorded as concomitant medications.

22 **Day 15** procedures will be performed in remot or on-site visit.

23 **End of study visit**: all subjects will undergo safety and efficacy follow-up assessments on Day 31 (±3 days) or earlier if patient withdraw from study.

Note: Central randomisation with IWRS system will be implemented using stratified permuted blocks to balance groups for stratification factors. Patients will be assigned to each arm at a 1:1:1 ratio. Baseline is Day 1 prior to start of the study treatment. Antiviral treatment to be used according to the approved product information in each country, different dosages could be used.

Appendix 6 - Contraception and Pregnancy Testing

This document is based on the Heads of Medicines Agencies' Recommendations Related to Contraception and Pregnancy Testing in Clinical Trials, published by the Clinical Trial Facilitation Group (CTFG) on 21 September 2020 and available at https://urldefense.com/v3/__https:/www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2020_09_HMA_CTFG_Contraception_guidance_Version_1.1_updated.pdf

A woman is considered of child-bearing potential (WOCBP) following menarche and until becoming postmenopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhoea, a single FSH measurement is insufficient. Immediately after detecting a case of suspected pregnancy in a patient, the decision on her continued participation in the clinical trial will be jointly taken by the patient, the Investigator and the Sponsor, with the patient's best interest in mind. A decision to continue the pregnancy will require immediate discontinuation of any investigational medicinal product (IMP).

A man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy. Fertile male patients included in this study should refrain from fathering a child or donating sperm during the study and for 6 months following the last IMP dose. If they have WOCBP partners the male subject should use condom during treatment and for 6 months following the last IMP dose.

Highly effective birth control methods are:

1. Combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[1]:
    a. oral
    b. intravaginal
    c. transdermal
2. Progestogen-only hormonal contraception associated with inhibition of ovulation[1]:
    a. oral
    b. injectable
    c. implantable[2]
3. Intrauterine device (IUD)[2]
4. Intrauterine hormone-releasing system (IUS)[2]
5. Bilateral tubal occlusion[2]
6. Vasectomised partner[2,3]
7. Sexual abstinence[4]
8. A combination of male condom with either cap, diaphragm, or sponge with spermicide (double barrier methods) are also considered acceptable, but not highly effective, birth control methods.

[1] Hormonal contraception may be susceptible to interaction with the IMP, which may reduce the efficacy of the contraception method (see below).
[2] Contraception methods that are considered to have low user dependency.
[3] Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success.
[4] Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject. Periodic abstinence (calendar, symptothermal, postovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not acceptable methods of contraception. Female condom and male condom should not be used together.

Contraception methods with low user dependency should preferably be used, in particular when contraception is introduced as a result of participation in this trial.

Appendix 7 – Barthel Index for Functional Assessment

	With help	Independent
1. Feeding (if food needs to be cut = help)	5	10
2. Moving from wheelchair to bed and return (includes sitting up in bed)	5-10	15
3. Personal toilet (wash face, comb hair, shave, clean teeth)	0	5
4. Getting on and off toilet (handling clothes, wipe, flush)	5	10
5. Bathing self	0	5
6. Walking on level surface (or if unable to walk, propel wheelchair) *score only if unable to walk	10 0*	15 5*
7. Ascend and descend stairs	5	10
8. Dressing (includes tying shoes, fastening fasteners)	5	10
9. Controlling bowels	5	10
10. Controlling bladder	5	10

Appendix 8 - Protocol for QTc Substudy

Evaluation of the Effect of Plitidepsin on Cardiac Repolarisation (QTc Duration) in Patients with COVID-19 Treated in Study APL-D-003-20

INTRODUCTION

Cardiac toxicity evaluation during preclinical/toxicology studies with plitidepsin did not suggest any potential clinically relevant cardiotoxicity risk in humans. A retrospective study of the cardiac adverse events (e.g., rhythm abnormalities, myocardial injury events, electrocardiogram [ECG] alterations) that occurred in patients (n=578) treated with single-agent plitidepsin was conducted. This study showed that the frequency of these events was not different from the frequency reported in the age-matched healthy population, thereby suggesting that plitidepsin- has a safe cardiac risk profile in cancer patients.[1]

This QT corrected (QTc) substudy associated with clinical trial APL-D-003-20 will determine whether plitidepsin at 1.5 and 2.5 mg flat dose on Days 1 to 3 has any effects on the QT interval in patients with coronavirus disease 2019 (COVID-19).

Substudy Rationale

An undesirable effect of some drugs is their ability to delay cardiac repolarisation, which can be measured as a prolongation of the QT interval on the surface ECG. The QT interval represents the duration of ventricular depolarisation and subsequent repolarisation, and is measured from the beginning of the QRS complex to the end of the T wave.

A delay in cardiac repolarisation creates an electrophysiological environment that favours the development of cardiac arrhythmias, mostly TdP but possibly other ventricular tachyarrhythmias as well. TdP is a polymorphic ventricular tachyarrhythmia that appears on the ECG as a continuous twisting of the vector of the QRS complex around the isoelectric baseline. The TdP can degenerate into ventricular fibrillation, leading to sudden death. Documented cases of TdP (fatal and nonfatal) associated with drug use have resulted in the withdrawal of several drugs from the market and the relegation of others to second-line status.

In general, there is a qualitative relationship between QT prolongation and the risk of TdP, especially for drugs that cause substantial prolongation of the QT interval. Therefore, an adequate pre-marketing investigation of the safety of a new pharmaceutical agent should include rigorous characterisation of its effects on the QT/QTc interval.[2]

Because of its inverse relationship to the heart rate, the measured QT interval is routinely corrected by several formulae to a less heart rate-dependent value, which is known as the QTc interval. Fridericia's correction method (QTcF)[3] is considered the more accurate general method for heart rate correction.[4] In certain cases, individual QT correction based on QT-RR relationship is a preferred method.[5]

Substudy Design Rationale

Patients with COVID-19 included in study APL-D-003-20 allows assessment in a Phase 3 clinical trial setting similar to that for which therapeutic use of plitidepsin is being evaluated. This substudy will be conducted at some of the sites participating in study APL-D-003-20.

Although the daily variability in QTc interval in these patients' setting has not been well described, it may be even larger because enrolled patients are likely to have advanced age or concomitant medical problems.[6] For this reason, an intrapatient standard deviation of 30 milliseconds is assumed. To reduce intrapatient variability due to measurement error, it has been recommended that the baseline QTc be rigorously investigated and be expressed as the mean of multiple ECG assessments.[7] For this purpose, 2 sets of triplicate ECG tracings recorded before plitidepsin administration will be considered baseline assessments: 1 set before and 1 set after prophylactic antiemetic medication administration.

Electrocardiogram assessments will be performed by means of continuous 12-lead ECG automated digital collection. All digital ECG continuous recordings will be sent to a third-party central ECG laboratory for measurement of intervals, diagnostics of abnormalities and review of ECG waveform morphology review. This will prevent the potential introduction of bias during the analysis of ECG recordings.

Samples for whole blood plitidepsin concentration measurement will be collected matching the clock times of ECG measurements, and are aimed at conducting a concentration QTc analysis.

OBJECTIVES

Based on the aforementioned scientific background, the primary objective of the present substudy is to assess the potential effects of plitidepsin at a therapeutic dose on the duration of the QTc interval, measured by continuous 12-lead ECG automated monitoring, in patients with COVID-19.

Secondary objectives are:

- To characterise the plitidepsin concentration/QTc relationship.
- To explore waveform morphology-related ECG parameters
- To explore plitidepsin pharmacokinetics in patients with COVID-19

OVERALL SUBSTUDY DESIGN

This QT substudy is to be performed in a subset of sites of the multicentre, randomised, Phase 3 clinical trial APL-D-003-20 conducted in patients with COVID-19.

The selection of sites will be made based on the site's feasibility to accomplish the QT evaluation study procedures.

Patients will be enrolled, as needed, to ensure that there are 50 evaluable patients in more than 80% of the scheduled ECG timepoints.

A continuous digital ECG recorder (Holter) will be provided by a third-party central ECG laboratory after the site has completed the site qualification requirements. Once the site has finished participation in the QT evaluation study, a recall notification will be sent to the investigator to return the equipment.

Study nurse and site personnel in charge of the QT substudy procedures will be trained and certified by means of 2 on-line courses about patient's preparation and use of the ECG equipment.

After completing all other baseline assessments on Day 1, a 12-lead continuous ECG recorder will be set in place. Patients will stay at the study site on Days 1 to 4 until completion of the pharmacokinetic (PK) blood sample collection scheduled at 24 hours after end of Day 3 infusion.

On Days 1, 2, and 3, administered the prophylactic medications to patients should be ideally completed 20 to 30 minutes before the start of infusion and, exceptionally, up to 2 h pre-infusion of plitidepsin.

Details on the timing of the treatment and assessments are given in the substudy Schedule of Assessments and Procedures (see below).

PATIENT DEFINITION

Substudy Population

Patients will be enrolled in the substudy as needed to ensure that approximately 50 patients have completed all required assessments.

Patient Eligibility

Inclusion Criteria

To be enrolled in the substudy, patients must satisfy the following criteria at screening for the main study:

1. Patient included in the clinical study APL-D-003-20 and randomised to the experimental arm (plitidepsin).
2. Patient with voluntarily signed and dated informed consent to participate in the QT substudy.
3. A 12-lead ECG consistent with normal cardiac conduction and function, showing:
    - Sinus rhythm.
    - Heart rate between 45 and 100 beats per minute.
    - QTcF ≤450 msec for males or ≤470 msec for females.
    - QRS interval <110 msec.
    - PR interval <200 msec.

4. Blood pressure between 90 and 150 mmHg systolic, inclusive, and not higher than 90 mmHg diastolic.
5. Serum electrolyte levels within 7 days before first infusion, Grade ≤ 1:
    - Ionic Ca++: 1.0 – 1.5 mmol/L.*
    - K+: 3 – 5.5 mmol/L.**
    - Mg++: 0.5 – 1.23 mmol/L.

* Corrected serum Ca for albumin = Measured serum Ca (mmol/l) +0.2 * [4.0 – Measured albumin (g/dl)].

** Patients with K <4 mmol/l could have their potassium repleted to allow them to participate. If so, K should be retested to confirm that new levels of serum K are within the specified range.

Exclusion Criteria

Patients who meet any of the following criteria will be excluded from participating in the substudy:

1. Age >65 years.
2. Has heart rhythm disturbances (eg atrial fibrillation), unusual T wave and U wave (if present) morphology, personal or family history of Long QT Syndrome, ECG findings of complete left bundle branch block, permanent ventricular pacemaker, or Brugada Syndrome.
3. Significant ischaemic coronary disease, New York Heart Association (NYHA) class III or IV congestive heart failure, myocardial infarction, or unstable angina within the last 6 months.
4. Has any skin condition likely to interfere with ECG electrode placement, or a history of breast implant or thoracic surgery likely to cause abnormality in electrical conduction.
5. Patients who at screening are on medication that is known to prolong the QT interval and/or induce TdP (rated as 1 in Appendix 8a), if it cannot be interrupted at least 48 hours before each ECG assessment.

Patient Registration

The patients who are willing to participate in substudy will have to sign QTc substudy-specific informed consent form (ICF). After the patient has signed the QTc substudy ICF and all eligibility criteria are met, the sponsor will confirm that patient can be included into this QT evaluation study. The patient number used throughout this QT evaluation study must be the same patient number allocated as part of the APL-D-003-20 study and this number should be used in all documentation and correspondence referring to this patient.

Completion/Withdrawal/Discontinuation from the QT Evaluation Study

A patient will be considered to be on QT study from the signature of the QTc substudy ICF to the removal of Holter on Day 3. If a patient does not receive treatment on Day 2,

the off-study date will be that day upon Holter removal. A patient will be withdrawn from the QT evaluation study for any of the following reasons:

- Not finally treated in the APL-D-003-20 study
- Patient's withdrawal of consent from the QT evaluation study
- Investigator's decision
- Noncompliance with inclusion and exclusion criteria or study procedures
- Major protocol deviation.

Patients who withdraw from the QT evaluation study (or who do not complete for whatever reason) will be replaced and/or recruitment continued as needed to ensure that there are approximately 50 patients in more than 80% of the scheduled ECG timepoints.

TREATMENT

Prophylactic Medication

During the substudy, all patients must be administered with the following prophylactic medications, which should be ideally completed 20 to 30 minutes before initiating the plitidepsin infusion and, exceptionally, up to 2 h before plitidepsin infusion start:

- Palonosetron 0.25 mg intravenous (IV; tropisetron 5 mg IV could be considered if palonosetron is not available).
- Diphenhydramine hydrochloride 25 mg IV (or equivalent such as dexchlorpheniramine maleate 5 mg).
- Ranitidine 50 mg IV (or equivalent, such as famotidine 20 mg IV).
- Dexamethasone phosphate 8 mg IV (equivalent to 6.6 mg dexamethasone base).

Additionally, to mitigate plitidepsin-induced nausea and vomiting, on Days 4 and 5 patients treated with plitidepsin should receive tropisetron 5 mg PO/IV if tropisetron 5 mg IV was administered on Days 1, 2, and 3.

Tropisetron 5 mg should be administered PO/IV on Days 4 and 5 if tropisetron 5 mg IV was administered on Days 1, 2, and 3.

The use of alternative prophylactic medications should be discussed in advance with the sponsor.

Prohibited Medications/Therapies

Drugs known to prolong QT interval and/or induce TdP are prohibited (rated as 1 in Appendix 8a). Patients must have been off these medications for a minimum of 48 hours before plitidepsin administration.

Ondansetron is a prohibited medication during the QT evaluation (Days 1 to 3) because it has been associated with a 6 msec QT interval prolongation.[8]

SUBSTUDY PROCEDURES

Pharmacokinetic Evaluations

All patients included in the study will be sampled for PK. Blood samples will be obtained on Day 1, 2, 3, and 4. All sample collection dates and times will be recorded on the eCRF.

Eleven blood samples will be collected at the timepoints detailed in Table 6 for the determination of whole blood concentrations of plitidepsin.

Table 6: Sampling Schedule for the Determination of Plitidepsin

Sample Number	Day	Absolute Time (h) From the Start of First Plitidepsin Infusion	Time-points for Plitidepsin Samples (Time Window)
1	1	0	Just before first infusion
2	1	1	5 min before EOI (±4 min)
3	1	2.5	1.5 h after EOI (±15 min)
4	1	5	4.0 h after EOI (±2 hour)
5	2	24	Just before Day 2 SOI (-15 to -1 min before SOI)
6	2	25	5 min before EOI (±4 min)
7	2	26.5	1.5h after EOI (±15 min)
8	2	29	4.0 h after EOI (±2 hour)
9	3	48	Just before Day SOI (-15 to -1 min before SOI)
10	3	49	Just before EOI (±4 min)
11	4	72	23 h after EOI (±4 h)

Abbreviations: EOI = end of infusion; h = hour; SOI = start of infusion

Samples for the measurement of plitidepsin will have a volume of 2 mL. The exact recording of the time of drug administration and sampling times is crucial on treatment days with PK sampling (and should be noted accurately even if not according to the planned time schedule). The infusion rate of plitidepsin will be established to ensure that the total dose is infused in 1 hour. The drug will be infused at a constant rate throughout the 1-hour period. To obtain reliable PK information, the infusion rate should not be modified once the infusion begins. If a variation in the infusion time does occur, it is important to reflect this in the eCRF, clearly writing the time of the beginning and the end of the infusion. The infusion rate should not be changed to maintain the scheduled duration of infusion. It would be enough to record the actual duration in the eCRF and on the PK sampling sheet.

Blood samples for PK will be obtained through a peripheral vein located in the contralateral side to that of administration of plitidepsin. In any case, the sampling vein has to be different from that in which drugs are administered. The last sample must never be collected from the catheter used for the drug administration.

If the blood sample is obtained from a catheter, the first millilitre (mL) of blood will be discarded to avoid the dilution of the sample with the solution used to keep it clean. Heparin (10 U/mL in normal saline solution) or a slow drip of normal saline solution (10 mL/h) can be used to keep the catheter permeable between extractions.

Samples should be collected in a sodium heparin tube and gently inverted several times to ensure proper mixing. After sampling, the test tubes containing the blood samples will be immediately frozen until analysis. Samples are NOT to be centrifuged.

Once all samples from a patient have been collected, they should be shipped to the designated laboratory as soon as possible, ideally the next shipping day. If the same centre has samples from several patients, samples can be sent in the same shipment. However, the time span between the moment the last PK sample for a patient has been

collected and the shipment of all samples from this patient to the laboratory should not exceed 2 months.

A manual of full instructions for sample extraction, labelling, storage, and shipment will be provided as a separate document.

ECG Evaluations

12-lead ECG Acquisition

The ECGs will be obtained digitally using a Mortara Instrument (Milwaukee, WI, USA) H-12+ECG continuous 12-lead digital recorder, which will obtain ECGs on Day 1 through Day 3. The ECGs will be stored continuously on a flash card and will not be available for review until the card is received by the emergency response team (ERT) and analysed. The patient's unique identification number and demographic information will be recorded for each card.

Patients will wear the continuous ECG recorder (Holter) from Days 1 to 3 (24 hours after end of infusion of Day 2). This ECG recorder will be provided by ERT after the study site has registered online at ERT's MYSTUDY PORTAL™. Registration details will be provided to the site coordinator at each participating site.

The ECG recorder will be set in place once all safety assessments scheduled for the main study (i.e., vital signs, blood sample extraction for laboratory tests, and ECG) have been conducted.

In this QT substudy, using pretreatment ECGs as the baseline and individual heart rate QT-correction, the Holter will be started, ideally, no less than 3 hours prior to the start of infusion of Day 1 through approximately 24 hours after the end of infusion of Day 2 (until the end of infusion on Day 3).

ECG Analysis Methods

All digital continuous ECG recordings will be sent to a third-party, central ECG laboratory ERT. The ECG readers will be blinded to patient identifiers and visit. All ECGs for a given patient will be analysed by the same reader. Quality Assurance reports for inter- and intra-observer variability will be produced by the central ECG laboratory and provided to the sponsor.

Serial ECGs with stable resting heart rate will be extracted from the continuous recording in triplicate (three 10-second digital ECGs in close succession) as close as possible to the following timepoints relative to Day 1 and Day 2: 5 minutes before the EOI (time-matched to PK sample #2 and #6, respectively), 1.5 (time-matched to PK sample #3 and #7), 4 (time-matched to PK sample #4 and #8), and 23 hours (time-matched to PK sample #5, and #9) after EOI. On Day 1, 2 sets (triplicate) of baseline ECGs; preprophylaxis (Predose 1) and postprophylaxis and before start of infusion (SOI) (Predose 2; time-matched to PK sample #1).

For the individual QT-RR correction (QTcI), low confidence beats from the continuous ECG recording will be reviewed using a pass-fail criterion.

Interval duration measurements will be collected using computer assisted caliper placements on 3 consecutive beats. Trained analysts will then review all ECGs for correct lead and beat placement and will adjudicate the preplaced algorithm calipers as necessary using the proprietary validated electronic caliper system applied on a computer screen. A cardiologist will then verify the interval durations and perform the morphology analysis, noting any T-U wave complex that is compatible with an effect on cardiac repolarisation.

The ECG analysis will be conducted in Lead II, or in Lead V5 if Lead II is not analysable. If Lead V5 is not analysable, then Lead V2 will be used followed by the most appropriate lead, if necessary.

On-screen measurements of the RR, PR, QRS, and QT interval durations will be performed and variables for QTcF and heart rate will be obtained by the following processes:

- Three (3) RR, PR, QRS and QT will be measured and mean RR interval, PR interval, QRS width and QT interval will be reported
- The following calculations will be made from the interval measurements:

Three (3) QTcF measurements (QTc correction by Fridericia)	Three (3) HR measurements
QTcF1 = QT1/3√RR1	HR1 = 60/RR1
QTcF2 = QT2/3√RR2	HR2 = 60/RR2
QTcF3 = QT3/3√RR3	HR3 = 60/RR3
Mean QTcF = (QTcF1+QTcF2+QTcF3)/3	Mean HR=(HR1+HR2+HR3)/3

Abbreviations: HR = heart rate; QTcF = QT interval corrected using Fridericia's formula

Each fiducial point (onset of P wave, onset of Q wave, offset of S wave, and offset of T wave) will be marked electronically. The original ECG waveform and such annotations will be saved separately in XML format for independent review.

For the triplicate ECGs collected at each timepoint, the average of the 3 measurements for each ECG parameter will be considered for all listing and statistical analyses described below.

Investigators are not expected to evaluate or otherwise assess the ECGs unless alerted by the central ECG laboratory to the possibility of a clinically relevant reading.

QT Correction Methods

The QT will be corrected for heart rate using the following methods:

- QTcF[3]
- Estimation of the exponent at an individual level (QTcI)[9]

The most appropriate method, based on the lack of statistical significance of the slope between QTc and heart rate, will be used for the concentration-QTc analysis.

Endpoint definition

In principle, for each patient and each postdose timepoint on Days 1 to 3, baseline QTc intervals will be the mean of the predose 1 and predose 2 QTc values for that patient on Day 1.

If the mean predose 2 QTc value of all evaluable patients is ≥10 msec longer than of the mean predose 1 QTc value, an effect of prophylactic medication on QTc cannot be ruled out; therefore, individual predose 2 QTc values will be considered as the baseline values.

The change from baseline (ΔQTc) will be calculated using the corresponding baseline QTc values. Specifically:

- ΔQTc at time t: Difference in QTc between scheduled timepoint t and baseline.

The primary endpoint will be ΔQTc at each scheduled timepoint.

STATISTICAL METHODS

Sample Size

Assuming that the intrasubject standard deviation for change from baseline in QTc (ΔQTc) is 30 msec and that the true difference between means is 5 msec, a sample size of at least 25 evaluable patients in more than 80% of the scheduled ECG timepoints *t* is planned to have 80% power to show that the upper limit of the 2-sided 90% confidence interval (CI; 1-sided upper 95% CI) for mean ΔQTc at each ECG timepoint *t* is <20 msec.

Primary Analysis

"By Timepoint" Analysis

The primary objective of this QT evaluation study is to measure the potential effect of plitidepsin on QTc. The primary comparison of interest is ΔQTc at each ECG timepoint t. A noninferiority criterion of 20 msec will be used to establish that postbaseline QTc is noninferior to baseline.

An analysis of variance model (ANOVA) with mixed effects will be fitted, with ΔQTc data as the dependent variable and ECG timepoint t as the fixed effects, and patient as random effect.

Using the estimated least square means (LSM) and intrapatient standard deviation obtained from this model, a 2-sided 90% CI will be calculated for each LSM ΔQTc. Noninferiority will be concluded if the upper bound of the 1-sided 95% CI falls below 20 msec at each ECG timepoint.

Mean QTc and corresponding changes from baseline (ΔQTc) will be listed and summarised descriptively by ECG timepoint. Plots of mean ΔQTc *versus* ECG timepoint will be prepared.

Categorical Analysis

Categorical (or outlier) reference limits will be defined in terms of change from baseline values or as absolute values, according to the ICH E14 guidance.

The incidence counts and percentage of patients with ΔQTc ≤30 msec, ΔQTc 30 to 60 msec and ΔQTc >60 msec will be tabulated for each ECG timepoint t and described for age and gender.

The incidence counts and percentage of patients with any new postbaseline QTc values >450 msec, >480 msec, and >500 msec will be tabulated for each ECG timepoint t, and described for age and gender. Patients with new QT and QTc values >500 msec will be listed.

Secondary Analyses

C-ΔQTc analysis

Exploratory Analysis

Before the model selection for the C-ΔQTc analysis, a potential delay of the ΔQTc effect relative to plasma concentrations (hysteresis) will be graphically and statistically assessed, as proposed by Darpo et al.[10] Plots of the time course of mean plasma concentrations and mean ΔQTF will be overlaid, to allow detection of delay in the time to QTc peak relative to plasma concentrations. In addition, study data will be analysed to check whether any of the following 3 statistical criteria are met to conclude the presence of hysteresis:

1. Largest mean ΔQTc exceeding 5 msec at ≥3 timepoints
2. Time difference between U_{max} (the time of the largest mean ΔQTc) and T_{max} of plitidepsin exceeding 1 hour, and
3. One-sided 1-sample Wilcoxon test for the difference between ΔQTc at T_{max} and at U_{max} formally significant at the 1% level

If the presence of hysteresis is concluded, the primary analysis of the C-ΔQTc will be based on an effect compartment ("indirect") model. However, if only some of the 3 criteria (not all) are met, the indirect model will be assessed and compared with a direct effect ("direct") model and, consequently, the model that provides the best description of the available data will be used for the primary model based analysis of the C-ΔQTc.

To enable an adequate comparison of the model fit between the direct and the indirect model, the model based predicted total plasma concentrations at central compartment (direct model) and at the effect compartment (indirect model) will be used.

Main Analysis

The relationship between total plasma concentration and ΔQTc will be assessed using a linear mixed effects (LME) model, as proposed by Garnett *et al.*[11] As the data available

come from a single arm study, the LME model will be characterised by the intercept (θ_1) and the slope (θ_2), and their corresponding variability (Equation 1, as below):

$$\Delta QTcF_{ij} = (\theta_1 \cdot e^{\eta_{1,i}}) + (\theta_2 \cdot e^{\eta_{2,i}}) \cdot C_{ij} + \theta_3 \cdot Day + e_{ij} \quad \text{Equation 1}$$

Where C_{ij} is the model-predicted plitidepsin total whole blood (observed) and or plasma (model-based estimated, as sensitivity analysis) concentrations (or the plitidepsin-concentrations in the effect compartment if a hysteresis is present), and $\eta_{1,i}$ and $\eta_{2,i}$ are the random effects associated with the intercept term θ_1 and the slope term θ_2, respectively, and are assumed to be exponential, independent and normally distributed. Furthermore, e_{ij} is the random residual variability, assumed to be an additive, independent, and normally distributed random variable. In the absence of placebo data, the unstructured placebo model consisting on the fixed effect parameters accounting for treatment-specific intercept (*TRT*), mean QTc change from baseline at each timepoint evaluated (*TIME*), and baseline QTc, will not be included in the model. The model-based prediction of the total plasma concentrations will be based on the individual PK parameters, obtained through a maximum *a posteriori* estimation based on the individual total plasma concentration available for each subject and a population PK model of plitidepsin, previously developed.[12] Based on the fact that ECG assessments in this study will be performed on 2 occasions (Days 1 and 2), the effect of Day θ_3 will also be assessed in the models.

The improvement in the data fit obtained for each model will be assessed by examining several diagnostics. The change in the minimum value of the objective function (MVOF), a statistic that is proportional to minus twice the log-likelihood of the data, will be examined. For hierarchical models, this change represents a statistic that is asymptotically distributed like χ^2, with degrees of freedom (DF) equal to the number of parameters added to or deleted from the model. A change in the MVOF (ΔMVOF) of 6.63 will be required to reach statistical significance at p = 0.01 for the addition of 1 fixed parameter.

Change in Other Parameters

Mean ECG parameters (heart rate, QRS, PR, and waveform morphology-related measurements) and corresponding changes from baseline will be listed and summarised descriptively, and plotted by ECG timepoint t.

The incidence counts of patients with new PR >200 msec, new QRS >120 msec, heart rate >100 or <50 beats per minute and a >25% change from baseline, will be tabulated for each ECG timepoint t, and described for age and gender.

Morphological Analysis

The incidence counts of patients with new abnormalities in the ST segment (i.e., depressed, elevated) and T wave (i.e., inverted) and U wave (presence of a new abnormal U wave) will be reported, as well as new findings of atrial fibrillation, atrial flutter, second degree or higher AV block, complete left or right bundle branch block or new myocardial infarction.

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Table 7: Schedule of Assessments and Procedures (QTc Substudy):

Procedure	Screening Day 0 (days)	Day 1							Day 2						Day 3				
		-3 hours (at least)	-30 min	predose	0	+1 hour (just before	+2.5 hours (1.5-h after EOI)	+5 hours (4-h after EOI)	-30 min	predose	0	+1 hours (just before	+2.5 hours (1.5-h after EOI)	+4 hours (5-h after EOI)	-30 min	predose	0	+1 hours (just before EOI)	+24 hours (23-h after EOI)
Written substudy-specific ICF	Before any procedures	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Review of substudy-specific inclusion/exclusion criteria	0 to 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Patient admission at the study site and Holter placement	-	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Intravenous prophylaxis medication	-	-	●	-	-	-	-	-	●	-	-	-	-	-	●	-	-	-	-
PK blood sample (2 mL)	-	-	-	●	-	●	●	●	-	●	-	●	●	●	-	●	-	●	●
Plitidepsin treatment	-	-	-	-	●	-	-	-	-	-	●	-	-	-	-	-	●	-	-

Abbreviations: EOI = end of infusion; ICF = informed consent form; PK = pharmacokinetic(s)

Appendix 8a: Lists of Drugs That Prolong the QT Interval and/or Induce Torsades de Pointes Ventricular Arrhythmia

Source: *www.azcert.org*

Because the evidence for risk of TdP is often imperfect, AZCERT, Inc. has divided the drugs into 4 groups based on their analysis of the evidence:

Risk of TdP: Substantial evidence supports the conclusion that these drugs prolong the QT interval AND are clearly associated with a risk of TdP, even when taken as directed in official labelling. In **bold** in the listing below.

Generic Name (Brand Name)	Drug Class/Clinical Usage	Risk List
Amiodarone (Cordarone®)	Antiarrhythmic/abnormal heart rhythm	**1**
Anagrelide (Agrylin®, Xagrid®)	Phosphodiesterase inhibitor/thrombocythaemia	**1**
Arsenic trioxide (Trisenox®)	Anticancer/leukaemia	**1**
Astemizole (Hismanal®)	Antihistamine/allergic rhinitis	**1**
Azithromycin (Zithromax®)	Antibiotic/bacterial infection	**1**
Bepridil (Vascor®)	Antianginal/heart pain	**1**
Chloroquine (Aralen®)	Antimalarial/malaria infection	**1**
Chlorpromazine (Thorazine®)	Antipsychotic/antiemetic/schizophrenia/nausea	**1**
Cilostazol (Pletal®)	Phosphodiesterase inhibitor/intermittent claudication	**1**
Cisapride (Propulsid®)	Gastrointestinal stimulant/heartburn	**1**
Citalopram (Celexa®)	Antidepressant/depression	**1**
Clarithromycin (Biaxin®)	Antibiotic/bacterial infection	**1**
Cocaine	Local anaesthetic	**1**
Disopyramide (Norpace®)	Antiarrhythmic/abnormal heart rhythm	**1**
Dofetilide (Tikosyn®)	Antiarrhythmic/abnormal heart rhythm	**1**
Domperidone (Motilium®)	Antinausea/nausea	**1**
Donepezil (Aricept®)	Cholinesterase inhibitor/Dementia	**1**
Dronedarone (Multaq®)	Antiarrhythmic/atrial fibrillation	**1**
Droperidol (Inapsine®)	Sedative;antinausea/anaesthesia adjunct, nausea	**1**
Erythromycin (E.E.S.®)	Antibiotic; gastrointestinal stimulant/bacterial infection; increase gastrointestinal motility	**1**
Escitalopram (Cipralex®)	Antidepressant/depression/anxiety disorders	**1**
Flecainide (Tambocor®)	Antiarrhythmic/abnormal heart rhythm	**1**
Halofantrine (Halfan®)	Antimalarial/malaria infection	**1**
Haloperidol (Haldol®)	Antipsychotic/schizophrenia, agitation	**1**
Ibutilide (Corvert®)	Antiarrhythmic/abnormal heart rhythm	**1**
Levomethadyl (Orlaam®)	Opiate agonist/pain control, narcotic dependence	**1**
Mesoridazine (Serentil®)	Antipsychotic/schizophrenia	**1**

Generic Name (Brand Name)	Drug Class/Clinical Usage	Risk List
Methadone (Methadose®)	Opiate agonist/pain control, narcotic dependence	**1**
Moxifloxacin (Avelox®)	Antibiotic/bacterial infection	**1**
Ondansetron (Zofran®)	Antiemetic/nausea and vomiting	**1**
Pentamidine (Pentam®)	Antiinfective/pneumocystis pneumonia	**1**
Pimozide (Orap®)	Antipsychotic/Tourette's tics	**1**
Probucol (Lorelco®)	Antilipemic/hypercholesterolaemia	**1**
Procainamide (Procan®)	Antiarrhythmic/abnormal heart rhythm	**1**
Quinidine (Quinaglute®)	Antiarrhythmic/abnormal heart rhythm	**1**
Sevoflurane (Ulane®)	Anaesthetic, general/anaesthesia	**1**
Sotalol (Betapace®)	Antiarrhythmic/abnormal heart rhythm	**1**
Sparfloxacin (Zagam®)	Antibiotic/bacterial infection	**1**
Sulpiride (Dogmatil®, Dolmatil®)	Antipsychotic, atypical/schizophrenia	**1**
Terfenadine (Seldane®)	Antihistamine/allergic rhinitis	**1**
Thioridazine (Mellaril®)	Antipsychotic/schizophrenia	**1**
Vandetanib (Caprelsa®)	Anticancer/thyroid cancer	**1**

Risk Level 2: Possible risk of TdP: Substantial evidence supports the conclusion that these drugs can cause QT prolongation BUT there is insufficient evidence at this time that these drugs, when used as directed in official labelling, are associated with a risk of causing TdP.

Risk Level 3: Conditional risk of TdP: Substantial evidence supports the conclusion that these drugs are associated with a risk of TdP BUT only under certain conditions (e.g., excessive dose, hypokalaemia, congenital long QT or by causing a drug-drug interaction that results in excessive QT interval prolongation).

Risk Levels 1 to 4: Drugs to Avoid in Congenital Long QT: Substantial evidence supports the conclusion that these drugs pose a risk of TdP for patients with congenital long QT. Drugs on this list include those in the above 3 risk categories 1, 2, and 3, and other drugs, 4, that do not prolong the QT interval per se but have a theoretical risk of causing arrhythmia that is based on their known stimulant actions on the heart.

Generic Name (Brand Name)	Drug Class/Clinical Usage	Risk List
Albuterol/salbutamol (Proventil®, Ventolin)	Bronchodilator/asthma	4
Alfuzosin (Uroxatral®)	Alpha1-blocker/benign prostatic hyperplasia	2
Amantadine (Symmetrel®)	antiviral/anti-infective/Parkinson's disease	3
Amphetamine (Adderal-XR®, Dexedrine®)	CNS stimulant/ADHD	4
Amiodarone (Cordarone®)	Antiarrhythmic/abnormal heart rhythm	**1**
Amisulpride (Solian® and others)	Antipsychotic, atypical/psychosis	3
Amitriptyline (Elavil®)	Tricyclic antidepressant/depression	3
Amoxapine (Asendin®, Amokisan®)	Tetracyclic antidepressant/depression	3
Anagrelide (Agrylin®, Xagrid®)	Phosphodiesterase inhibitor/thrombocythaemia	**1**
Apomorphine (Apokyn®, Ixense®)	Dopamine agonist/Parkinson's disease	2

Generic Name (Brand Name)	Drug Class/Clinical Usage	Risk List
Aripiprazole (Abilify®, Aripiprex®)	Antipsychotic/psychosis, adjunct for depression	2
Arsenic trioxide (Trisenox®)	Anticancer/leukaemia	**1**
Arformoterol (Brovana®)	Bronchodilator/chronic obs. lung disease	4
Astemizole (Hismanal®)	Antihistamine/allergic rhinitis	**1**
Atazanavir (Reyataz®)	Protease inhibitor/HIV	2
Atomoxetine (Strattera®)	Norepinephrine reuptake inhibitor/ADHD	4
Azithromycin (Zithromax®)	Antibiotic/bacterial infection	**1**
Bedaquiline (Sirturo®)	Anti-infective/drug-resistant tuberculosis	2
Bepridil (Vascor®)	Antianginal/heart pain	**1**
Bortezomib (Velcade®, Bortecad®)	Proteasome inhibitor/multiple myeloma,lymphoma	2
Bosutinib (Bosulif®)	Tyrosine kinase inhibitor/leukaemia	2
Chloral hydrate (Noctec®)	Sedative/sedation/insomnia	3
Chloroquine (Aralen®)	Antimalarial/malaria infection	**1**
Chlorpromazine (Thorazine®)	Antipsychotic/antiemetic/schizophrenia/nausea	**1**
Cilostazol (Pletal®)	Phosphodiesterase inhibitor/intermittent claudication	**1**
Ciprofloxacin (Cipro®)	Antibiotic/bacterial infection	3
Cisapride (Propulsid®)	Gastrointestinal stimulant/heartburn	**1**
Citalopram (Celexa®)	Antidepressant/depression	**1**
Clarithromycin (Biaxin®)	Antibiotic/bacterial infection	**1**
Clomipramine (Anafranil®)	Tricyclic antidepressant/depression	3
Clozapine (Clozaril®)	Antipsychotic/schizophrenia	2
Cocaine	Local anaesthetic	**1**
Crizotinib (Xalkori®)	Kinase inhibitor/anticancer	2
Dabrafenib (Tafinlar®)	Anticancer/melanoma	2
Dasatinib (Sprycel®)	Tyrosine kinase inhibitor/leukaemia	2
Desipramine (Pertofrane®)	Tricyclic antidepressant/depression	3
Dexmedetomidine (Precedex®, Dexdor®)	Sedative/sedation	2
Dexmethylphenidate (Focalin®)	CNS stimulant/ADHD	4
Dextroamphetamine (Dexedrine®)	CNS stimulant/ADHD	4
Dihydroartemis.+piperaquine (Eurartesim®)	Antimalarial/malaria	2
Diphenhydramine (Benadryl®)	Antihistamine/allergic rhinitis, insomnia	3
Disopyramide (Norpace®)	Antiarrhythmic/abnormal heart rhythm	**1**
Dobutamine (Dobutrex®)	Inotrope/heart failure, shock	4
Dopamine (Intropine®)	Inotrope/heart failure, shock	4
Dofetilide (Tikosyn®)	Antiarrhythmic/abnormal heart rhythm	**1**
Dolasetron (Anzemet®)	Antinausea/nausea, vomiting	2
Domperidone (Motilium®)	Antinausea/nausea	**1**

Generic Name (Brand Name)	Drug Class/Clinical Usage	Risk List
Donepezil (Aricept®)	Cholinesterase inhibitor/dementia	**1**
Doxepin (Sinequan®)	Tricyclic antidepressant/depression	3
Dronedarone (Multaq®)	Antiarrhythmic/atrial fibrillation	**1**
Droperidol (Inapsine®)	Sedative;antinausea/anaesthesia adjunct, nausea	**1**
Ephedrine (Rynatuss®, Broncholate®)	Bronchodilator, decongestant/allergies, asthma	4
Epinphrine (Primatene®, Bronkaid®)	Vasoconstrictor/Anaphilaxis, allergic reactions	4
Eribulin (Halaven®)	Anticancer/metastatic breast neoplasias	2
Erythromycin (E.E.S.®)	Antibiotic; gastrointestinal stimulant/bacterial infection; increase gastrointestinal motility	**1**
Escitalopram (Cipralex®)	Antidepressant/depression/anxiety disorders	**1**
Famotidine (Pepcid®)	H2-receptor antagonist/peptic ulcer/gastroesophageal reflux disease	2
Felbamate (Felbatrol®)	Anticonvulsant/seizure	2
Fenfluramine (Pondimin®, Ponderax®)	Appetite suppressant/Dieting, weight loss	4
Fingolimod (Gilenya®)	Immunosuppressant/multiple sclerosis	2
Flecainide (Tambocor®)	Antiarrhythmic/abnormal heart rhythm	**1**
Fluconazole (Diflucan®)	Antifungal/fungal infection	3
Fluoxetine (Sarafem®)	Antidepressant/depression	3
Formoterol (Foradil, Foralide)	Bronchodilator /Asthma	4
Foscarnet (Foscavir®)	Antiviral/HIV infection	2
Fosphenytoin (Cerebyx®)	Anticonvulsant/seizure	2
Furosemide/Frusemide (Lasix®, Fusid®)	Diuretic/increase urine & salt loss	3
Galantamine (Reminyl®)	Cholinesterase inhibitor/dementia, Alzheimer's	3
Gatifloxacin (Tequin®)	Antibiotic/bacterial infection	2
Gemifloxacin (Factive®)	Antibiotic/bacterial infection	2
Granisetron (Kytril®)	Antinausea/nausea and vomiting	2
Halofantrine (Halfan®)	Antimalarial/malaria infection	**1**
Haloperidol (Haldol®)	Antipsychotic/schizophrenia, agitation	**1**
Hydrochlorothiazide (Apo-Hydro®)	Diuretic/increase urine & salt loss	3
Ibutilide (Corvert®)	Antiarrhythmic/abnormal heart rhythm	**1**
Iloperidone (Fanapt®)	Antipsychotic, atypical/schizophrenia	2
Imipramine (Norfranil®)	Tricyclic antidepressant/depression	3
Indapamide (Lozol®)	Diuretic/stimulate urine & salt loss	3
Isoproterenol (Medihaler-Iso®, Isuprel®)	Bronchodilator/allergic reaction	4
Isradipine (Dynacirc®)	Antihypertensive/high blood pressure	2
Itraconazole (Sporanox®)	Antifungal/fungal infection	3
Ivabradine (Procoralan®, Coralan®)	Antianginal/angina pectoris (heart pain)	3
Ketoconazole (Nizoral®)	Antifungal/fungal infection	3

Generic Name (Brand Name)	Drug Class/Clinical Usage	Risk List
Lapatinib (Tykerb®)	Anticancer/breast cancer, metastatic	2
Levalbuterol (Xopenex®, Levolin®)	Bronchodilator/Asthma	4
Levofloxacin (Levaquin®)	Antibiotic/bacterial infection	2
Levomethadyl (Orlaam®)	Opiate agonist/pain control, narcotic dependence	**1**
Lisdexamfetamine (Vyvanse®)	CNS stimulant/ADHD	4
Lithium (Lithobid®)	Antimania/bipolar disorder	2
Mesoridazine (Serentil®)	Antipsychotic/schizophrenia	**1**
Metaproterenol (Metaprel®, Alupent®)	Bronchodilator/asthma	4
Methadone (Methadose®)	Opiate agonist/pain control, narcotic dependence	**1**
Methamphetamine (Desoxyn®, Pervitin®)	CNS stimulant/Obesity, ADHD	4
Methylphenidate (Ritalin®, Concerta®)	CNS stimulant/ADHD	4
Metronidazole (Flagyl®)	Antibiotic/trichomoniasis, amebiasis, bacterial infection	3
Midodrine (ProAmatine®, Amatine®)	Vasoconstrictor/Low blood pressure, fainting	4
Mifepristone (Korlym®, Mifeprex®)	Progesterone antagonist/pregnancy termination	2
Mirabegron (Myrbetriq®)	Beta3 adrenergic antagonist/overactive bladder	2
Mirtazapine (Remeron®)	Antidepressant/depression	2
Moexipril/HCTZ (Uniretic®)	Antihypertensive/high blood pressure	2
Moxifloxacin (Avelox®)	Antibiotic/bacterial infection	**1**
Nelfinavir (Viracept®)	Antiviral/HIV/AIDS	3
Nicardipine (Cardene®)	Antihypertensive/high blood pressure	2
Nilotinib (Tasigna®)	Anticancer/leukaemia	2
Norepinephrine (Levophed ®)	Vasoconstrictor/Shock, low blood pressure	4
Norfloxacin (Noroxin®, Ambigram®)	Antibiotic/bacterial infection	2
Nortriptyline (Pamelor®)	Tricyclic Antidepressant/depression	3
Ofloxacin (Floxin®)	Antibiotic/bacterial infection	2
Olanzapine (Zyprexa®)	Antipsychotic, atypical/schizophrenia, bipolar	2
Ondansetron (Zofran®)	Antiemetic/nausea and vomiting	**1**
Oxytocin (Pitocin®)	Oxytocic/labour stimulation	2
Paliperidone (Invega®)	Antipsychotic, atypical/schizophrenia	2
Paroxetine (Paxil®)	Antidepressant/depression	3
Pasireotide (Signifor®)	Somatostatin analogue/Cushing's Disease	2
Pazopanib (Votrient®)	Tyrosine kinase inhibitor/anticancer	2
Pentamidine (Pentam®)	Anti-infective/pneumocystis pneumonia	**1**
Perflutren lipid microspheres (Definity®)	Imaging contrast agent/echocardiography	2
Phentermine (Adipex P®, Adiphene®)	Appetite suppressant/Dieting, weight loss	4
Phenylephrine (Neosynephrine®)	Vasoconstrictor/Low blood pressure, allergies	4
Phenylpropanolamine (Acutrim, Dexatrim®)	Appetite suppressant/Obesity	4

Generic Name (Brand Name)	Drug Class/Clinical Usage	Risk List
Pimozide (Orap®)	Antipsychotic/Tourette's tics	**1**
Pipamperone (Dipiperon®, Propitan®)	Antipsychotic/schizophrenia	2
Posaconazole (Noxafil®, Posamol®)	Antifungal/fungal infection	3
Probucol (Lorelco®)	Antilipemic/hypercholesterolemia	**1**
Procainamide (Procan®)	Antiarrhythmic/abnormal heart rhythm	**1**
Promethazine (Phenergan®)	Antipsychotic-antiemetic/nausea	2
Protriptyline (Vivactil®)	Tricyclic antidepressant/depression	3
Pseudoephedrine (PediaCare®, Sudafed®)	Decongestant/allergies, sinusitis, asthma	4
Quetiapine (Seroquel®)	Antipsychotic/schizophrenia	2
Quinidine (Quinaglute®)	Antiarrhythmic/abnormal heart rhythm	**1**
Quinine sulfate (Qualaquin®)	Antimalarial/malaria or leg cramps	3
Ranolazine (Ranexa®)	Antianginal/chronic angina	2
Rilpivirine (Edurant®, Complera®)	Antiviral/HIV/AIDS	2
Risperidone (Risperdal®)	Antipsychotic/schizophrenia	2
Ritodrine (Yutopar®)	Muscle relaxant/Prevent premature labour	4
Ritonavir (Norvir®)	Protease inhibitor/HIV	3
Roxithromycin* (Rulide®)	Antibiotic/bacterial infection	2
Saquinavir (Invirase®)	Antiviral/HIV/AIDS	2
Salmeterol (Serevent®, Advair®)	Bronchodilator/Asthma, COPD	4
Sertindole (Serdolect®)	Antipsychotic, atypical/anxiety, schizophrenia	2
Sertraline (Zoloft®)	Antidepressant/depression	3
Sevoflurane (Ulane®)	Anaesthetic, general/anaesthesia	**1**
Sibutramine (Meridia®)	Appetite suppressant/Dieting, weight loss	4
Solifenacin (VESIcare®)	Muscarinic receptor antagonist/overactive bladder	3
Sorafenib (Nexavar®)	Tyrosine kinase inhibitor/anticancer	2
Sotalol (Betapace®)	Antiarrhythmic/abnormal heart rhythm	**1**
Sparfloxacin (Zagam®)	Antibiotic/bacterial infection	**1**
Sulpiride (Dogmatil®, Dolmatil®)	Antipsychotic, atypical/schizophrenia	**1**
Sunitinib (Sutent®)	Anticancer/RCC, GIST	2
Tacrolimus (Prograf®)	Immunosuppressant/immune suppression	2
Tamoxifen (Nolvadex®)	Anticancer/breast cancer	2
Telaprevir (Incivek®, Incivo®)	Antiviral/hepatitis C	3
Telavancin (Vibativ®)	Antibiotic/bacterial infection	2
Telithromycin (Ketek®)	Antibiotic/bacterial infection	2
Terbutaline (Brethine®, Bricanyl®)	Bronchodilator/Asthma, premature labour	4
Terfenadine (Seldane®)	Antihistamine/allergic rhinitis	**1**
Tetrabenazine (Nitoman®, Xenazine®)	Monoamine transporter inhibitor/chorea	2
Thioridazine (Mellaril®)	Antipsychotic/schizophrenia	**1**

Generic Name (Brand Name)	Drug Class/Clinical Usage	Risk List
Tizanidine (Zanaflex®)	Muscle relaxant/spasticity	2
Tolterodine (Detrol®, Detrusitol®)	Muscle relaxant/bladder spasm	2
Toremifene (Fareston®)	Oestrogen agonist/antagonist/anticancer	2
Trazodone (Desyrel®)	Antidepressant/depression, insomnia	3
Trimethoprim-Sulfa (Septra® or Bactrim®)	Antibiotic/bacterial infection	3
Trimipramine (Surmontil®)	Tricyclic antidepressant/depression	3
Vandetanib (Caprelsa®)	Anticancer/thyroid cancer	**1**
Vardenafil (Levitra®)	Phosphodiesterase inhibitor/vasodilator	2
Vemurafenib (Zelboraf®)	Kinase inhibitor/anticancer	2
Venlafaxine (Effexor®)	Antidepressant/depression	2
Voriconazole (Vfend®)	Antifungal/fungal infection	3
Vorinostat (Zolinza®)	Anticancer/lymphoma	2
Ziprasidone (Geodon®)	Antipsychotic/schizophrenia	2

A note about brand names: drugs are listed with up to two common brand names. There are many more brand names for some of the common drugs, such as pseudoephedrine and erythromycin.

Appendix 9 - Imputation of PaO_2 from SpO_2

Patients with severe COVID-19 are not considered eligible for this study. The FDA Guidance "*COVID-19: Developing Drugs and Biological Products for Treatment or Prevention Guidance for Industry*"[1] includes an assessment of the PaO_2/FiO_2 ratio, to discriminate between 'Moderate' and 'Severe' categories.
However, direct measurement of PaO_2 is considered an invasive procedure and is rarely used in this patient population. Instead, most patients will only have determinations of oxyhemoglobin percent saturation with a pulse oximeter (SpO_2). Therefore, it will be necessary to implement a method for the estimation of the PaO_2/FiO_2 ratio to evaluate disease severity in those patients for whom an arterial gasometry is contemplated inappropriate. The following two approaches will be considered valid for the purpose of patient eligibility, as they are the result of an appropriate validation methodology:

- The Ellis inversion[2] (Figure 1) of the Severinghaus equation[3] provides a useful nonlinear method for imputing PaO2 from SaO_2. This technique has been used in cohorts of mostly nonintubated patients with pneumonia[4-6] and in patients with acute respiratory distress syndrome[7].

$$PO_2 = \left\{ \frac{11{,}700}{\left(1/S - 1\right)} + \left[50^3 + \left(\frac{11{,}700}{1/S - 1} \right)^2 \right]^{1/2} \right\}^{1/3} + \left\{ \frac{11{,}700}{\left(1/S - 1\right)} - \left[50^3 + \left(\frac{11{,}700}{1/S - 1} \right)^2 \right]^{1/2} \right\}^{1/3}$$

Fig 1. Ellis solution for the Severinghaus equation
(S= SpO_2 from pulse oximetry)

- Other groups using data from patients with acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) who were enrolled in two large clinical trials, found that SpO_2/FiO_2 ratio correlated well with a simultaneously obtained PaO_2/FiO_2 ratio, and could serve as a surrogate for the latter. The regression formula is as follows[8]:

$$\frac{SpO2}{FiO2}(\%) = 64 + 0.84 * \frac{PaO2}{FiO2}(\%)$$

where:

$$\frac{PaO2}{FiO2}(\%) = \left[\frac{SpO2}{FiO2}(\%) - 64\right] * 1.19$$

Fig 2. Rice et al PaO_2/FiO_2 imputation from SpO_2/FiO_2

In addition they showed that SpO_2/FiO_2 ratios of 235 and 315 were found to correspond to PaO_2/FiO_2 ratios of 200 and 300, respectively, which are the oxygenation criteria defining ARDS and ALI, respectively.[9] These threshold SpO_2/FiO_2 ratios demonstrated excellent sensitivity and good specificity in predicting the corresponding PaO_2/FiO_2 ratios in a validation data set.

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Appendix 10 - Adjustment of PaO_2 from a Site at High Altitude

For sites at high altitude, it is very important to correct the PaO_2/FIO_2 by altitude or barometric pressure (i.e., raise the value of PaO_2/FIO_2 obtained at altitude), but unfortunately, the adjustment is not a simple function of altitude or barometric pressure. At the same shunt, PaO_2/FIO_2 decreases with altitude, impairing a proper comparison of individuals with similar lung damage if they reside at different altitudes and if the shunt is not directly measured, as is the case in most patients.

In the well-known Berlin definition of acute respiratory distress syndrome (ARDS)[1], there is a recommended adjustment for arterial oxygen partial pressure to fractional inspired oxygen (PaO_2/FIO_2) at altitude, but without a reference as to how it was derived. In acclimatised patients undergoing invasive mechanical ventilation, this traditional equation for adjusting PaO_2/FiO_2 according to the elevation above the sea level seems to be inaccurate.[2] The correction suggested by the working group does the opposite of what is needed, because it requests a multiplication by a fraction < 1 (barometric pressure (Pbar)/760).

The following formula was obtained computationally, and tested on a group of patients with ARDS secondary to pneumonia, admitted during 2014-2015 to the intensive care unit of a referral center for respiratory diseases in the metropolitan area of Mexico City (altitude 2,240 m above sea level).[3]

Estimate PaO_2/FIO_2 at sea level (PaO_2/FIO_2SL)

$$\frac{PaO2}{FiO2}SL = \left(\frac{PaO2}{FiO2} * 1.245\right) + (FiO2 * 51.51) + (0.0307 * altitude) - 88$$

Where PaO_2/FIO_2 is the actual ratio determined at the site or imputed from the actual SpO_2/FiO_2 ratio (according to Appendix 9), and altitude is expressed in meters.

These proposed adjustments appropriately raise the PaO_2/FIO_2 measured at altitude, so that the same sea level cut-point limits to classify ARDS severity can be utilised when comparing patients living at different altitudes above sea level. (3)

References

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Appendix 11 - Age-adjusted Charlson Index

This Index is calculated by the sum of weights associated with co-morbidities and age[1]:

- Conditions with a weight of 1:
  - myocardial infarction,
  - congestive heart failure,
  - peripheral vascular disease,
  - cerebrovascular disease,
  - dementia,
  - chronic pulmonary disease,
  - connective tissue disease,
  - ulcer disease,
  - mild liver disease,
  - diabetes.
- Conditions with a weight of 2:
  - hemiplegia,
  - moderate or severe renal disease,
  - diabetes with end organ damage
  - any malignancy.
- Conditions with a weight of 3:
  - Moderate or severe liver disease (e.g., cirrhosis with ascites)
- Conditions with a weight of 6:
  - Metastatic solid tumour or AIDS
- Each decade of age over 40 adds 1 point to risk, i.e,,
  - Age between 50-59 years: add 1 point;
  - Age between 60-69 years: add 2 points;
  - Age between 70-79 years: add 3 points;
  - Age between 80-89 years: add 4 points;
  - Age between 90:99 years: add 5 points;
  - Age between 100-109 years: add 6 points

Reference

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Appendix 12 - Imputation of FiO_2 from oxygen flow values. High-flow oxygen therapy.

Oxygen delivery systems are categorised into low-flow and high-flow systems.When the patient inspires, the oxygen is diluted with room air, and the degree of dilution depends on the inspiratory flows. Therefore, these oxygen delivery systems do not allow for accurate calculation of the inspiratory oxygen fraction (FiO_2).

There is no perfect solution to estimate the FiO_2 delivered by a low-flow device. It depends on many factors, such as the type of device, the respiratory minute volume, factors related to the delivery site (nose and/or mouth, trachea), etc. For the purpose of this trial, we will follow the conversion described below in the table, as it is one of the most widely used both in medical practice and clinical research[1]:

Imputation of FiO_2 from the information on the device and oxygen flow prescribed:

100% O_2 Flow Rate (L/min)	FiO_2 (%)
Nasal Cannula / Prongs	
1	24
2	28
3	32
4	36
5	40
6	44
Oxygen Mask	
5-6	40
6-7	50
7-8	60
Mask with Reservoir Bag	
6	60
7	70
8	80
9	90
10	>99

High-flow oxygen delivery systems provide higher oxygen flows and the FiO_2 is stable and is not affected by the patient's type of breathing. Following FDA guidelines[2], the definition of "**high flow oxygen therapy**" that fulfills the criteria for respiratory failure is the delivery of heated, humidified, oxygen via reinforced nasal cannula at flow rates > 20 L/min with a fraction of delivered oxygen ≥ 0.5.

References:

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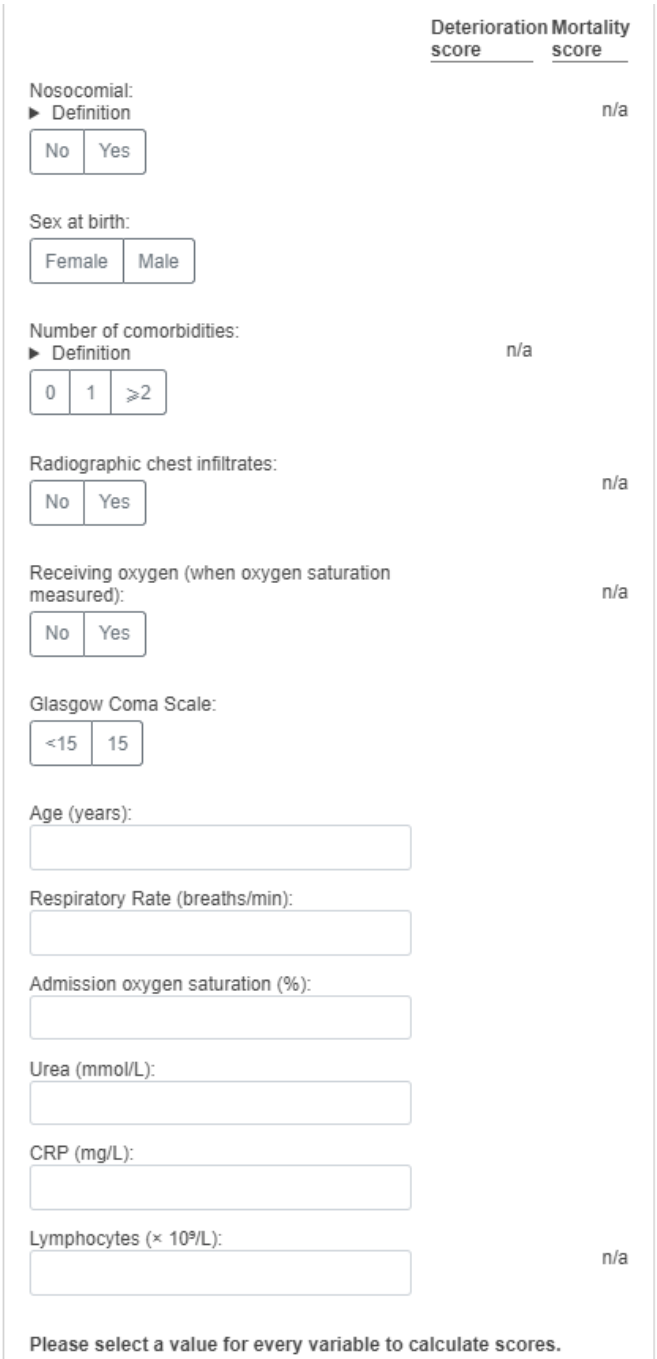
Appendix 13 - World Health Organization (WHO) Clinical Progression Scale

Patient State	Descriptor	Score
Uninfected	Uninfected; no viral RNA detected	0
Ambulatory mild disease	Asymptomatic; viral RNA detected	1
	Symptomatic; independent	2
	Symptomatic; assistance needed	3
Hospitalised: moderate disease	Hospitalised; no oxygen therapy*	4
	Hospitalised; oxygen by mask or nasal prongs	5
Hospitalised: severe diseases	Hospitalised; oxygen by NIV or high flow	6
	Intubation and mechanical ventilation, $pO_2/FiO_2 \geq 150$ or $SpO_2/FiO_2 \geq 200$	7
	Mechanical ventilation $pO_2/FIO_2 < 150$ ($SpO_2/FiO_2 < 200$) or vasopressors	8
	Mechanical ventilation $pO_2/FiO_2 < 150$ and vasopressors, dialysis, or ECMO	9
Dead	Dead	10

Abbreviations: ECMO = extracorporeal membrane oxygenation; FiO_2 = fraction of inspired oxygen; NIV = non-invasive ventilation; pO_2 = partial pressure of oxygen; RNA = ribonucleic acid; SpO_2 = oxygen saturation
* If hospitalised for isolation only, record status as for ambulatory patient

Appendix 14 – ISARIC 4C Mortality & 4C Deterioration Scores*



*As data is no primarily collected and as it is a exploratory study, urea will be transformed form BUN and a Glasgow equal to 15 will be assumed for all the patients.

Source: https://isaric4c.net/risk/

Appendix 15 –Glucocorticoids equivalent anti-inflammatory doses

The conversion to equivalent doses between different corticoids is described with some variability. For the purpose of this study we will use the concept of anti-inflammatory equipotency to calculate the equivalences between drugs.

Corticosteroid	Equivalent Anti-inflammatory doses[1,2]	Equipotent dose (mg) to Dexamethasone (base) 6 mg
Cortisol (hydrocortisone)	20	160
Cortisone	25	200
Deflazacort[3]	7.5	60
Prednisone	5	40
Prednisolone	5	40
6-methylprednisolone	4	32
Triamcinolone	4	32
Betamethasone	0.6	4.8
Dexamethasone	0.75	6

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