Protocol Number: AV-APL-B-002-22

Official Title: A Multicentre, Open label, Randomised, Controlled, Basket, Pragmatic, Phase II, Clinical and Translational Study to Determine the Efficacy and Safety of Plitidepsin versus Control in Immunocompromised Adult Patients with Symptomatic COVID-19 requiring Hospital Care (NEREIDA)

NCT Number: NCT05705167

Document Date: 22 March 2023

Pharma Mar S.A.

Protocol Number: AV-APL-B-002-22 **ICON Study: PAMAAPL1-AVAPL1**

EudraCT number: 2022-002489-34 **CONFIDENTIAL**

SPONSOR
Pharma Mar, S.A.
Avda. de los Reyes, 1
Polígono Industrial "La Mina"
28770 Colmenar Viejo (Madrid) Spain
Phone: + 34 91 846 6000
Fax: + 34 91 846 6003

CLINICAL TRIAL PROTOCOL

AV-APL-B-002-22

A Multicentre, Open label, Randomised, Controlled, Basket, Pragmatic, Phase II, Clinical and Translational Study to Determine the Efficacy and Safety of Plitidepsin *versus* Control in Immunocompromised Adult Patients with Symptomatic COVID-19 requiring Hospital Care (NEREIDA)

INVESTIGATIONAL MEDICINAL PRODUCTS: Plitidepsin.

Protocol Code: AV-APL-B-002-22

EudraCT No: 2022-002489-34

Version 3.0 - 22 MAR 2023

Sponsor's Medical Contact: [redacted]

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and the applicable regulatory requirements.

Confidentiality statement

Information and data included in this protocol contain trade secrets and privileged or confidential information which is the property of the Sponsor. No person is authorized to make it public without written permission of the Sponsor. These restrictions on disclosure will apply equally to all future information supplied to you which is indicated as privileged or confidential. This material may be disclosed to and used by your staff and associates as it may be necessary to conduct the clinical study.

SPONSOR APPROVAL

I have read the following and approve it:

_____ _____

[redacted]

Date

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SYNOPSIS

TITLE	A Multicentre, Open label, Randomised, Controlled, Basket, Pragmatic, Phase II, Clinical and Translational Study to Determine the Efficacy and Safety of Plitidepsin *versus* Control in Immunocompromised Adult Patients with Symptomatic COVID-19 requiring Hospital Care (NEREIDA).	
PROTOCOL CODE	**AV-APL-B-002-22**	
INVESTIGATORS	A full list of Investigators will be available as a separate document.	
NUMBER OF SITES / TRIAL LOCATION	More than 50 sites are planned to participate in this study worldwide.	
STUDY OBJECTIVES and ENDPOINTS	**Objectives** **Primary objective** *Efficacy primary objective* To evaluate efficacy of plitidepsin in pre-specified groups of immunocompromised patients with symptomatic COVID-19 requiring hospital care *vs* control in terms of mortality.	**Endpoints** *Efficacy primary endpoint* **1-month[†] all-cause mortality rate.** [†](Day 30 since randomisation).
	Secondary objectives *Key efficacy secondary objective* To compare efficacy of plitidepsin *vs* the control in terms of viral clearance, in each group.	 *Efficacy secondary endpoint* Time to confirmed negativisation in SARS-CoV-2 antigen test or RT-PCR Ct > 30.

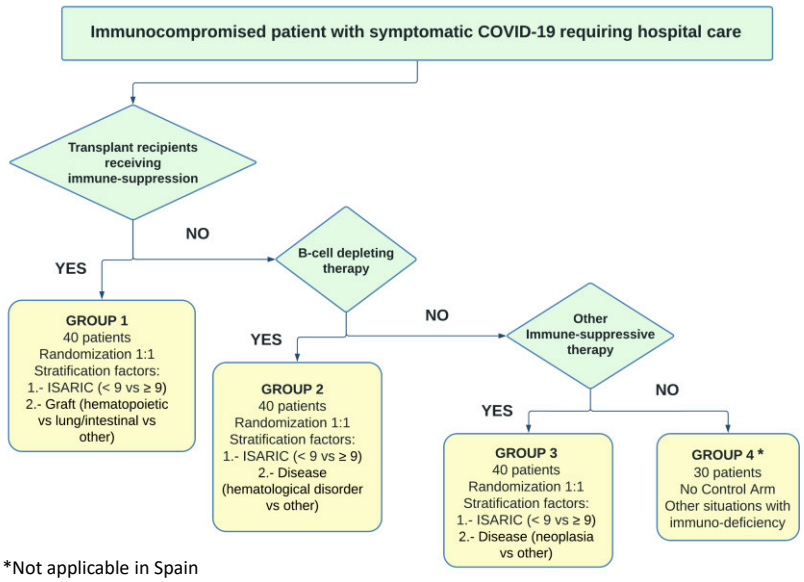
	Efficacy secondary objectives	*Efficacy secondary endpoints*
	To compare efficacy of plitidepsin *vs* the control in terms of sustained end of hospital care, in each group.	Time to sustained end of COVID-related hospital care from the time of randomisation. Sustained discharge is defined as no subsequent admission within 30 days of initial end of hospital care [up to day 60 (±3)], related to either COVID-19 or COVID-19 therapy.
	To compare efficacy of plitidepsin *vs* the control in terms of symptomatic improvement, in each group.	Time to sustained improvement (defined in Section 7.1.3) and resolution of selected COVID-19 signs/symptoms (See Appendix 10 - COVID-19 signs/symptoms checklist).
	To compare efficacy of plitidepsin *vs* the control in terms of clinical status (11-category WHO Clinical Progression Scale), in each group.	Distribution of patients according to their clinical status by the 11-category WHO Clinical Progression Scale, at Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3) (See Appendix 1 – 11-Point World Health Organization (WHO) Clinical Progression Scale).
	To compare efficacy of plitidepsin *vs* control in terms of the need of any kind of supplementary oxygen, in each group.	Percentage of patients requiring oxygen therapy on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3). Time to sustained discontinuation (i.e., at least 7 days) of oxygen supplementation.
	Safety secondary objectives	*Safety secondary endpoints*
	To compare safety/tolerability of plitidepsin *vs* the control in terms of adverse events, adverse reactions and mortality, in each group.	Frequency of the following events (all-cause and drug-related): • 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-related Serious Adverse Events (i.e., SARs); • Adverse events leading to treatment discontinuation; and • Deaths (COVID-19-related/all).
	To compare safety/tolerability of plitidepsin *vs* the control in terms of abnormal laboratory parameters, in each group.	Change respect to baseline* in individual study-defined laboratory parameters (See Section 5.7, 5.8 and 5.9 and Appendix 8 - Clinical Laboratory Analyses).
	To compare safety/tolerability of plitidepsin *versus* the control in terms of variations of vital signs, in each group.	Change respect to baseline* in individual vital signs (See Section 5.7, 5.8 and 5.9).
	Other secondary objectives	
	To compare efficacy of plitidepsin *vs* the control in the need of any type of advanced oxygen support (high-flow nasal oxygen, extracorporeal membrane oxygenation (ECMO), or non-invasive or invasive mechanical ventilation), in each group.	Percentage of patients requiring advanced oxygen support on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2,) and 60 (±3). Time to intensification of respiratory support (WHO >5) (See Appendix 1 – 11-Point World Health Organization (WHO) Clinical Progression Scale). Total duration of advanced oxygen support.
	To compare efficacy of plitidepsin *vs* the control in the need of intensification of respiratory or intensive care support, in each group.	Percentage of patients requiring high-flow oxygen on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3). Total duration of high-flow oxygen therapy per patient. Percentage of patients requiring non-

		invasive mechanical ventilation on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3). Total duration of non-invasive mechanical ventilation per patient. Percentage of patients requiring invasive mechanical ventilation or ECMO on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3). Total duration of invasive mechanical ventilation or ECMO per patient. Percentage of patients requiring admission to ICU on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3). Total duration of intensive care unit (ICU) stay.
	To compare efficacy of plitidepsin *vs* the control in the need of intensification of pharmacological therapies for COVID-19, in each group.	Time to onset of additional (i.e., not present at baseline) immune-modulating drugs. Percentage of patients receiving immune-modulating drugs on Days 1, 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3). Time to onset of additional (i.e., not present at baseline) antiviral drugs. Percentage of patients receiving subsequent antiviral drugs (i.e., not present at baseline) on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).
	To compare efficacy of plitidepsin *vs* control in terms of superinfection, in each group.	Percentage of patients with a new infection by Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).
	To compare efficacy of plitidepsin *vs* the control in terms of all-cause- and	Cumulative mortality (all-cause and related to COVID-19) by Days 4 (±1), 8

	related to COVID-19-mortality, in each group.	(±1), 15 (±1), 30 (±2), and 60 (±3).
	To compare efficacy of plitidepsin *vs* the control in terms of subsequent hospital admissions, in each group.	Percentage of patients in each study group who require subsequent admission within 30 days of initial end of hospital care [up to day 60 (±3)], related to either COVID-19 or COVID-19 therapy.
	To compare efficacy of plitidepsin *vs* the control in terms of the time course of viral load, in each group.	Change respect to Day 1 in the viral load of SARS-CoV-2, measured by RT-PCR, by Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), 45 (±2), and 60 (±3), in each study group. Percentage of patients in each study group with undetectable viral load, by Days 4 (±1), 8 (±1), 15 (±1), 45 (±2), 30 (±2), and 60 (±3). Time to either undetectable viral load of SARS-CoV-2 or > 2 logs reduction respect to Day 1.
	To compare efficacy of plitidepsin *vs* the control in terms of the evolution of inflammatory markers, in each group.	Change respect to baseline* in inflammatory biomarkers and immunological biomarkers (C-reactive protein [CRP], procalcitonin, lactate dehydrogenase [LDH], ferritin, neutrophil-to-lymphocyte ratio, ALC, D-dimer, and multiplex cytokines assay, by Day 2 (±1), 4 (±1), 8 (±1), 15 (±1) and 30 (±2) and 60 (±3).
	To compare efficacy of plitidepsin *vs* the control in terms of the immune response against SARS-CoV-2, in each group.	Change respect to baseline* in individual serological assessments against SARS-CoV-2, by Days 30 (±2), and 60 (±3). Change respect to baseline* in individual T-cell response against SARS-CoV-2 by Days 30 (±2), and 60 (±3).
	To compare efficacy of plitidepsin *vs* the control in	Change respect to baseline* to Days 15 (±1), and 30 (±2) in chest X-ray findings

	terms of radiological evolution, in each group.	(Brixia score, centrally assessed) (Appendix 9 – Brixia Score).
	To compare efficacy of plitidepsin *vs* the control in terms of restoration of the therapy for the underlying disease, in each group.	Percentage of patients requiring modification of the therapy (drugs, dose or schedule) for the underlying disease. Time in which pre-scheduled therapies for the control of the underlying disease were not able to be administered.
	To compare efficacy and safety/tolerability of all pooled plitidepsin arms *vs* all control arms in all the aforementioned endpoints.	All the aforementioned endpoints.
	To compare efficacy and safety/tolerability between plitidepsin arms (across different groups).	All the aforementioned endpoints.
	To explore prognostic/predictive factors for clinical deterioration or mortality or drug response.	Risk for mortality / clinical deterioration according to individual clinical characteristics and laboratory observations present at baseline*, scores of validated scales (e.g., ISARIC-4C), as well as features related to COVID-19 [including SARS-CoV-2 variant (if available), vaccination status, duration of the infection, and inflammation-related parameters [neutrophil-to-lymphocyte ratio (NLR), protein C reactive, interleukine-6 (IL-6) and lymphocytes], the underlying disease, and other co-morbidities.
	Increase pharmacology knowledge of plitidepsin.	Limited-sampling pharmacokinetics assessment (See Section 7.6). PK-PD analysis.
	*Baseline = latest test performed between screening and drug administration.	

STUDY DESIGN	This is a multicentre, randomised, controlled, open-label, pragmatic, phase 2 basket study to assess the efficacy and safety of plitidepsin in different groups of immunocompromised patients with symptomatic coronavirus disease 2019 (COVID-19) who require hospital care. Approximately 150 patients are foreseen for the study, divided in 4 different groups of immunocompromised patients: • **Group 1** – Patients receiving immune-suppression due to hematopoietic or organ transplantation**.** • **Group 2** – Patients receiving B-cell depleting therapies. • **Group 3** – Patients receiving other immune-suppressive therapies. • **Group 4*** – Other situations with immune deficiencies. *Not applicable in Spain Whenever a patient can be allocated to more than 1 group, the priority will be group 1 > group 2 > group 3 > group 4*. Patients will be randomised to receive best standard care (BSC) ± other antiviral (if clinically indicated) on control arm and BSC + plitidepsin on experimental arm. Group 4* will not be controlled.  *Not applicable in Spain

	Stratification: • Group 1: ISARIC mortality (<9/≥9) | Type of graft (allogeneic haematopoietic/lung or intestinal/other). • Group 2: ISARIC mortality (<9/≥9) | Disease (haematological neoplasm/other). • Group 3: ISARIC mortality (<9/≥9) | Disease (neoplasia/other). • Group 4*: Not applicable. *Not applicable in Spain **Study periods:** The study will include a Screening period, a Treatment period and a Follow-up period. The Screening period starts once the patient has provided written informed consent and ends when the patient is randomised in the study. Parameters assessed during this time will serve as the baseline values. The screening procedures should be preferably performed within 24 h prior to administration of study treatment, allowing a maximum of 48 h prior to administration of study treatment if required. The Treatment period starts on Day 1 (date of randomisation) to 24 h after the last dose of the antiviral therapy. If no antiviral was given in the control arm, then the treatment period will finish on day 4, for data management purposes. Antiviral treatment must always be started on the randomisation date. The Follow-up period starts after the end-of-treatment and lasts until the end-of-study. The End of Study (EOS) is Day 60 (±3), or date of early study termination unless ongoing SAEs, if applicable. The End of Trial (EOT) will be when the Last Patient Last Visit (LPLV) has occurred. All patients will receive the best available supportive care as per applicable local, institutional, national, supranational COVID-19 treatment guidelines. Patients assigned to the experimental arms will receive, in addition to BSC, plitidepsin as a 60-min IV infusion, during days 1 to 3. Patients randomised to the control arm may receive a regulatory-approved antiviral treatment in addition to the BSC.

	For prevention of infusion-related (i.e., plitidepsin-related) reactions (IRRs), allergic reactions and emesis reactions, administration of premedications is mandatory in the experimental arms. All consenting patients discontinuing early from the study before the EOS visit should complete the early termination/withdrawal procedures. **Independent Data Monitoring Committee (IDMC)** 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 not only to the study Sponsor but also to the 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. The 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/efficacy 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 60), to determine if there is a difference between plitidepsin and control groups and potential impact on study conduct. • Each group will proceed with per protocol pre-established recruitment and follow-up and 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 $\geq$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 to assess the continuity of the other groups. The IDMC can ask for a temporary halt of the clinical trial at any time to better analyse any potential benefit/risk concern. **Study "Stopping rules"** The Sponsor may suspend or terminate the study if: • New toxicological or pharmacological findings or safety issues that invalidate the earlier positive benefit-risk assessment occur; • Significant safety concerns, according to the Sponsor's criteria, arise during the conduct of the study; • The development of plitidepsin is discontinued; • Any other reason (e.g., best interest of the patients, accrual rate makes the completion of the study feasible, etc.) leads to this suspension or termination.
STUDY POPULATION	Immunocompromised adult patients with symptomatic COVID-19 requiring hospital care.
STUDY POPULATION **Inclusion criteria**	To be eligible for inclusion into this study, each patient must fulfil the following inclusion criteria within 48 hours prior to randomisation: **General inclusion criteria:** 1. Signed informed consent obtained prior to initiation of any study-specific procedures and study treatment; 2. Patient aged ≥18 years; 3. Patient diagnosed COVID-19, with the following characteristics: a) A regulatory approved test, collected no more than 3 days prior to study randomisation, with either a Ct value ≤30 or a positive antigen test; b) Presence of any of the selected signs/symptom listed in Appendix 10 - COVID-19 signs/symptoms checklist within the last 24 h; 4. Patient already admitted or requiring hospital care* for symptomatic COVID-19, for which at least one antiviral has failed†, or cannot be used‡, after a minimum washout period of 24h for small molecules (e.g., remdesivir, molnupiravir, nirmaltrevir/ritonavir) and 5 days for

antiviral monoclonal antibodies (e.g., tixagevimab + cilgavimab) or convalescent plasma.

*The definition of hospital care is based on the need to use a hospital environment (hospital ward, day hospital) for treatment administration and/or clinical monitoring of the patient with COVID-19.

†Failure of a prior antiviral is defined as a documented lack of clinical response, plus evidence of persisting positivity for SARS-CoV-2 in an appropriate biological sample, determined by a regulatory approved test, collected no more than 3 days prior to study randomisation, with either a Ct value ≤ 30 (RT-PCR) or a positive antigen test.

‡Contraindication, absence of labelled indication, guidelines or drug unavailability.

5. 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 ≥ 50 000/mm^3 (50 x 10^9/L);
   - Alanine transaminase (ALT) ≤3 x upper limit of normal (ULN) (≤5 x ULN if pre-existent liver involvement by the underlying disease);
   - Serum bilirubin ≤1.5 x ULN (or direct bilirubin <1.5 x ULN when total bilirubin is above ULN);
   - Estimated glomerular filtration rate ≥30 mL/min [CKD-EPI Creatinine Equation (2021)].

6. Females of childbearing potential must have a negative serum or urine pregnancy test by local laboratory at study enrolment and must be non-lactating.

7. Females of child-bearing potential must use highly effective contraceptive methods, while on study treatment and for 6 months after last dose of plitidepsin. Fertile males with partners of child-bearing potential must use effective contraception, while on study treatment and for 6 months after last dose of plitidepsin (See Appendix 2 – Contraception and pregnancy testing). Patients in the control arm must follow contraception methods 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 highly effective contraception (females of child-bearing potential) and effective (fertile males with partners of child-bearing potential) for at least one week after the study completion or the time indicated based on the investigator's discretion. **Group-specific inclusion criteria:** • **Group 1 – Patients receiving, within the last 30 days, immune-suppressive therapy due to haematopoietic or organ transplantation.** o Haematopoietic transplantation. o Solid Organ Transplantation: ▪ Lung / intestinal. ▪ Other. • **Group 2 – Patients receiving B-cell depleting therapies within the last 6 months*.** Includes (but is not limited to): o Monoclonal antibodies (mAbs) targeting CD19, CD20, CD38, or CD52 (e.g., rituximab, ocrelizumab, ofatumumab, daratumumab, alemtuzumab). o B-cell activating factor (BAFF) inhibitors (e.g., belimumab). o Bruton's tyrosine kinase (BTK) inhibitors (e.g., evobrutinib, ibrutinib). o Chimeric antigen receptor T cell therapy (CAR-T) (e.g., anti-CD19 CAR-T cell). *Time restriction is not applicable for CAR-T cell therapy. • **Group 3 – Patients receiving, within the last 30 days, other immune-suppressive therapies.** o Other immunosuppressive therapies not including B-cell depleting agents for the treatment of auto-immune disorders. o Chemotherapy or targeted therapies with immunosuppressive potential for solid tumours or haematological disorders. o Chronic glucocorticoids (i.e., equivalent to prednisone ≥ 20 mg/day for more than 1 month). • **Group 4* – Other situations with immunodeficiency**. Includes (but is not limited to): o Primary immune deficiencies.

	○ Human immunodeficiency virus (HIV) infection, with $CD4^+$ T lymphocytes < 200 cells/µL in the last month. ○ Radiation therapy within the last 3 months- requires documentation of ALC < 500 cells/µL. ○ Haematological neoplasia or myelodysplasia not currently receiving any therapy. ○ Other situations with a documentation of ALC < 500 cells/µL. *Not applicable in Spain Further information about groups and immunosuppressive therapies can be found in Appendix 12 – Definition of NEREIDA Study Groups and lists of immunosuppressive treatments.
Exclusion criteria	1. Evidence of critical illness, defined by at least one of the following: • Respiratory failure defined based on resource utilization requiring at least one of the following: endotracheal intubation and mechanical ventilation, ECMO, or clinical diagnosis of severe acute respiratory distress syndrome with PaO_2*/FiO_2 ≤ 100. *In case a direct measure of PaO_2 has not been obtained, it should be imputed according to a referenced formula (Ellis or Rice) (Appendix 5 - Imputation of PaO_2/FiO_2 ratio). For sites located over 1000 m altitude above sea level, PaO_2/FiO2 ratio will be adjusted (Appendix 6- Adjustment of PaO_2 from a Site at High Altitude; See also Appendix 7 for FiO2 imputations from oxygen flow rates). • Shock requiring vasopressors. • Multi-organ dysfunction/failure. 2. (Criterion eliminated and merged with inclusion criterion #4, based on AEMPS recommendations). 3. Any of the following cardiac conditions or risk factors: • Cardiac infarction or cardiac surgery episode within the last month; • History of known congenital QT prolongation; • Known structural cardiomyopathy with abnormal LVEF (<50%); • Current clinical evidence of heart failure or acute cardiac ischaemia (New York Heart Association (NYHA) class III-IV).

	4. Hypersensitivity to the active ingredient or any of the excipients (mannitol, macrogolglycerol hydroxystearate, and ethanol) or contraindication to receive dexamethasone, antihistamine H1/H2, or anti-serotoninergic $5HT_3$ agents. 5. Females who are pregnant or breast-feeding. 6. Females and males with partners of childbearing 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. 7. Any situation currently requiring increasing needs of immune suppressive agents (e.g., acute graft rejection, flare of autoimmune disorder, or cytokine storm syndrome). 8. 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. 9. Participation in another clinical study involving an investigational drug within 30 days prior to screening.
EXPECTED NUMBER OF PATIENTS	Approximately 150 are foreseen to be enrolled in the study.
REPLACEMENT OF PATIENTS	Patients will not be replaced, but accrual in each group will continue until the target sample size of patients evaluable for the primary endpoint is reached.
STUDY DRUGS FORMULATION	Powder for concentrate for solution for infusion.
ROUTE OF ADMINISTRATION	Intravenous (IV) infusion.
TREATMENT SCHEDULE AND DOSE	Plitidepsin will be administered as a 60-min (±5 min) IV infusion, every 24 h (±30 min) for 3 consecutive days, at a dose of 2.5 mg.

PROPHYLACTIC MEDICATION	For prevention of plitidepsin related infusion reactions, the following premedications MUST be given to each patient and should be completed no more than ~2 hours to 20 minutes before initiating each plitidepsin infusion: • Dexamethasone phosphate* 8 mg IV (equivalent to 6.6 mg of dexamethasone base); • Dexchlorpheniramine maleate* 5 mg IV (or diphenhydramine hydrochloride 25 mg IV); • Famotidine* 20 mg IV (or equivalent such as ranitidine 50 mg IV). Oral famotidine is acceptable, but in this case the dose should be 40 mg and it must be administered 2 hours (±15 min) before plitidepsin; • Palonosetron* 0.25 mg IV (tropisetron 5 mg IV could be considered if palonosetron is not available. It should be administered orally (PO)/IV on Days 4 and 5 if tropisetron 5 mg was administered on Days 1, 2 and 3). *Provided by the Sponsor, who is also in charge of the labeling (except when the site uses its own medication for the trial).
ALLOWED MEDICATIONS/ THERAPIES	Any medications, with the exceptions noted below and in Section 6.3.2 , which are considered necessary for the patient's welfare, and which will not interfere with the study medication, may be given at the discretion of the Investigator. Medications which MUST be administered as part of the study treatment schedule are listed in Section 6.1.5. Administration of **all concomitant drugs** received by the patient during the study must be reported in the appropriate section of the eCRF along with dosage information, dates of administration and reasons for use. Additionally, any unplanned diagnostic, therapeutic or surgical procedure performed during the study period must be recorded in the eCRF.
PROHIBITED MEDICATIONS/ THERAPIES	• Other investigational agents. • Concomitant use of plitidepsin and antiviral treatments for COVID-19. • Strong CYP3A4 inhibitors and inducers throughout plitidepsin treatment period and until 24-h washout period (See Appendix 3

	Inhibitors and Inducers of CYP3A4), unless medical judgement that they are for the patient's best interest. Investigator may decide to stop the study treatment at any time after the first dose of plitidepsin and initiate other pharmacological treatments for COVID-19.
DRUG-DRUG INTERACTIONS WITH PLITIDEPSIN	**Interactions with CYP3A4 inhibitors and inducers**: *In vitro* studies indicate that CYP3A4 is the major enzyme involved in plitidepsin metabolism, followed by CYP2A6 and CYP2E1. However, a pharmacokinetic population analysis of plitidepsin including 283 patients indicated that concomitant administration of CYP3A4 inhibitors and inducers does not affect exposure to plitidepsin. Nevertheless, it cannot be ruled out that concomitant treatments that strongly inhibit or induce CYP3A4 may modify the efficacy and/or increase the probability of side effects associated with plitidepsin, so they should be avoided. • Co-administration with CYP3A4 strong inhibitors and inducers should be avoided, as they may affect the plasma concentration of plitidepsin, unless medical judgement that they are for the patient's best interest. • Co-administration with CYP3A4 moderate inhibitors and inducers should be used with caution, as an effect on plitidepsin exposure cannot be excluded. A list of CYP3A4 inducers and inhibitors is included in Appendix 3 - Inhibitors and Inducers of CYP3A4.
DOSE REDUCTION	No dose modifications are foreseen at the experimental arm.
SAFETY EVALUATION CRITERIA	Safety parameters/covariates include: • Demographic Data including gender and age; • Medical history and prior medications and procedures; • ISARIC 4C Mortality & 4C Deterioration scores (Appendix 4); • AEs will be monitored throughout the study, which will start immediately after the patient Informed Consent Form (ICF) is signed;

	• Pregnancy; • Concomitant medications and procedures; • Physical examination including height and weight. Body Mass Index will be calculated; • Vital signs include body temperature (°C), systolic blood pressure (SBP) (mmHg), diastolic blood pressure (DBP) (mmHg), pulse rate (bpm) and respiratory rate (breaths/min); • Laboratory tests include haematology, coagulation and biochemistry; • Urinalysis; • Electrocardiograms (ECGs) include overall assessment, heart rate (HR), PR interval (PR), QRS duration (QRS), uncorrected QT interval (QT), and corrected QT interval by Fredericia (QTcF), and will be performed at the study site.
EFFICACY EVALUATION CRITERIA	Efficacy parameters include: • Mortality (all-cause and related to COVID-19); • COVID-19 signs and symptoms will be collected daily from Day 1 until the end of hospital care; thereafter at Days 4, 8 (±1), 15 (±1), 30 (±2), 45 (±2), and 60 (±3); • Clinical status is assessed using the WHO 11-point Clinical Progression Scale; • Lung function tests include fraction of inspired oxygen (FiO_2) (%), oxygen saturation (SpO_2), partial pressure of oxygen (PaO_2) (mmHg) [could be imputed from oxygen saturation (SpO_2)]. PaO_2/FiO_2 ratio will also be calculated (See Appendix 5 - Imputation of PaO_2/FiO_2 ratio); • SARS-CoV-2 quantitative real-time-reverse transcription polymerase chain reaction test (qRT-PCR) will be performed by central laboratory from oro-nasopharyngeal exudates on Day 1 before administration of the study drug and repeated at Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), 45 (±2), and 60 (±3). Name of the variant will be collected if known; • SARS-CoV-2 Rapid Antigen test (RAT) will be performed every other day from Day 4 until discharge, and always at Days 1, 4 (±1), 8 (±1), 15 (±1), 30 (±2), 45 (±2), and 60 (±3); if RAT is positive at discharge, RAT should also be monitored on a weekly basis until negative. A

	negative result should be confirmed with a second RAT within 48 h, by the investigator team; • Humoral response anti-SARS-CoV-2; • T-cell response against SARS-CoV-2; • Additional therapies for COVID-19; • Type and dates of oxygen support; • Radiological scores (chest X-ray, Brixia score; centrally assessed) (See Appendix 9 – Brixia Score); • Death date and reason; • Dates of discharge from ICU or hospital, and date of eventual re-admission; • Inflammatory and immunological biomarkers, including C-reactive protein (CRP), procalcitonin, lactate dehydrogenase (LDH), ferritin, neutrophil-to-lymphocyte ratio, ALC, D-Dimer, and multiplex cytokines assay; • Compliance with scheduled therapies for the underlying disease.
PHARMACOKINETIC (PK) EVALUATIONS	PK analysis of plitidepsin concentration-time data will be performed in 30 patients using non-linear mixed-effects modelling. Individual PK parameters will be (See Section 7.6): • Plasma total area under the curve (AUC). • Plasma total clearance (CL).
STATISTICAL METHODS	This is an exploratory basket study to adequately approach the heterogeneity of clinical conditions included under the concept of immune compromise. The study implements a Bayesian methodology. This decision has been adopted after taking into account the relative low frequency of the conditions under study, the relative absence of prospective data of the outcomes of COVID-19 for the study populations (most of the available data are retrospective and belong to the early waves of the pandemic, with different viral variants than nowadays), along with the changing medical practices in these settings. Under these premises, a Bayesian approach is more appropriate in terms of efficient decision-making process and will

	provide novel information for future therapeutic development in these clinical conditions. The trial will use a minimallyinformative prior of N(0,1) to update the information provided by the trial data to produce a posterior distribution. The resultant posterior distribution will serve as a starting point for further trials within this area, whether as a key part of any information data packs provided to experts ahead of an elicitation meeting, or directly as the informed prior or hypotheses for further trials. A calibrated Bayesian hierarchical model (CBHM) described by Chu and Yuan will be used to analyse the data, allowing data to be analysed by group, and results from each group will be used to enrich the data of the other groups. The calibration of the model allows strong information sharing when the treatment effect is similar among baskets and shrinks that sharing towards 0 as the treatment effect becomes more heterogeneous. An overall type I error of 5% will be used to analyse by means of credible regions the OR against a null of OR=1 [log(OR)=0], the case where no discernible difference is induced from intervention. Simulation was used to perform sensitivity analysis on a range of informed priors against a minimally informative prior to ensure that its use would not impact the results on the data too far. The statistical methods for this study are described in a detailed statistical analysis plan (SAP). **SAMPLE SIZE** **A) Randomised groups (1-3)** Patients in three of the four groups will be randomly assigned to one of two arms. Each randomised group will have a sample size of 40 patients (20 in the experimental arm, 20 in the control arm). Within the CBHM simulations, 5000 Markov-chain Monte Carlo (MCMC) repetitions per scenario were simulated with a range of operating characteristics for the null, alternative and true mortality rates. A maximum sample size of 40 per randomised group was set through the CBHM simulations, which in turn determines whether there is a need for the total sample size to be allocated. These simulations cover mortality rates between 5 and 95%. Within all simulations, the full 120 patients were used, suggesting that this is the minimum sample size that enables decision-making. As an example, if the mortality rate in each group is 20% and we target a clinically meaningful reduction of 12.5% in the experimental arm (H_0=20%, H_1=7.5%), and the assumed true mortality rate is 7.5%, the simulation demonstrates

	that a sample size of 40 per basket will allow for a posterior probability around 0.9 in each basket (i.e. 90% probability that the experimental arm is better than the control in each basket), ranging from 0.87 (when the data from the fastest basket is analysed) to 0.915 (at the end of the accrual of the last basket) (type I error rate= 0.05). **B) Non-controlled group 4*** Many different clinical conditions cannot be classified into one of the 3 randomised groups and have been pooled into a fourth group of 30 patients. As there is a remarkable intragroup heterogeneity, this group will not be controlled and will contribute to the objective of increasing safety knowledge of plitidepsin. . A Fleming's Phase II procedure with a 1-stage design has been used to estimate the sample size needed to rule out that plitidepsin induces drug-related SAEs in at least 35% of the patients (null hypothesis), and to confirm that the respective rate is 10 % or lower (alternative hypothesis), with a type I error of 0.05 and a power of 90%. If at least 25 patients out of a total sample of 30 do not experience a drug-related SAE, the null hypothesis will be rejected. *Not applicable in Spain **METHODS OF ANALYSIS** Analysis of the primary and other time-to-event efficacy endpoints will be performed primarily in the FAS but also in the "Per Protocol" and "As treated" populations. Supportive analyses will be performed on the Per Protocol population. Safety endpoints analyses will be based on the "As treated" Population. **Primary endpoint** The difference between treatment and control arms in all-cause mortality at 30 days will be examined using a Bayesian comparison of proportions within the calibrated Bayesian hierarchical model (CBHM) framework described by Chu and Yuan. The use of the CBHM allows for flexible information borrowing across all groups, with strong borrowing in the case when treatment effect is homogeneous among the groups and less borrowing as the effect becomes more heterogeneous. In this framework the odds ratio is modelled as $\theta i=\log(oddsi)$ with a normal distribution of unknown mean (μ) and variance ($\sigma 2$). The prior distribution used by the framework will be a minimally informative prior using N(0.1). The variance ($\sigma 2$) represents the degree of heterogeneity between the patient groups, with 0 representing the case where there is homogeneity of

	treatment effect and thus complete pooling of the results across patient groups, with adjustment for targeted p1 rates in each group, whereas a value closer to infinity would lead to no borrowing across groups. These estimates are iteratively updated within the modelling procedure based on the available data. As each basket is completed, they will be evaluated for efficacy, with information gained being used to update the analyses of successive baskets. Once all patients recruited to a basket have completed the trial, a formal analysis will be performed on the patients within the basket. The result will be able to stand on its own for the specific subpopulation, and the information from this analysis will be used to enrich the information at analysis of the proceeding baskets. Results in later baskets will be related to pooled data, however if one basket is not seen to be homogeneous to other baskets, the information from that basket can be run without the pooled information from other baskets and its information not pulled through into later analyses. As a sensitivity analysis the Bayesian hierarchical model will be used. The cut-off for considering further clinical development of plitidepsin in each randomised group will be a posterior probability of superiority for the experimental arm equal to or greater than 65%. **Secondary endpoints/Other endpoints** Frequency tables will be prepared for categorical variables, and continuous variables will be described by means of summary tables, which will include the median, mean, standard deviation, interquartile range, minimum, and maximum of each variable. In addition to Bayesian methods, frequentist methods will be also calculated. Counts and percentages, with their corresponding exact confidence intervals, will be calculated for the binomial endpoints. Time-to-event variables and their set time estimates will be analysed according to the Kaplan-Meier method and Cox regressions. Safety analyses will include AEs, SAEs, deaths, laboratory evaluations and study drug discontinuations due to AEs will be tabulated in a descriptive way. Pooled and subgroup analyses will be performed, taking into consideration baseline characteristics of the patients and of the underlying disease, risks factors for clinical deterioration or death, vaccination status,duration of the infection prior to study entry, and concomitant therapies (e.g.,

	immunomodulatory agents —including glucocorticoids—, oxygen supplementation).
DURATION OF STUDY PERIOD (per patient)	• **Screening period**: From the day of ICF signing prior to randomisation). • **Day 1:** Date of randomisation. Treatment must be started on the same date. • **Treatment period**: from Day 1 to 24 h after the last dose of the antiviral therapy; if no antiviral was given in the control arm, then the treatment period will finish on Day 4, for data management purposes. • **Follow-up period**: From the end-of-treatment to end-of-study. • **EOS**: Day 60 (±3) or date of early study termination unless ongoing SAEs, if applicable. • **Overall study duration**: 60 (±3) days since randomisation.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

5-HT3	Serotonin
AE(s)	Adverse Event(s)
AEMPS	Spanish Agency of Medicines and Medical Devices
AESI	Adverse Events of Special Interest
ALC	Absolute Lymphocyte Count
ALI	Acute Lung Injury
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AP	Alkaline Phosphatase
APTT	Activated Partial Thromboplastin Time
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BAFF	B-cell Activating Factor
βhCG	beta Human Chorionic Gonadotropin
bpm	Beats per minute
BSC	Best Standard Care
BTK	Bruton's Tyrosine Kinase
BUN	Blood Urea Nitrogen
CAR-T	Chimeric Antigen Receptor T-cell Therapy
CDHM	Calibrated Bayesian Hierarchical Model
CC_{50}	50% of maximum cytotoxic effect
CCL	Chronic Lymphocytic Leukemia
CD	Cluster of Differentation
CI	Confidence Interval
CL	Clearance
COVID-19	Coronavirus Disease 2019
CPK	Creatine Phosphokinase
CrCL	Creatinine Clearance
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeat
CRO	Contract Research Organisation
CRP	C-reactive Protein
CRX	Chest X-ray

Ct	Cycle Threshold
CTCAE	Common Terminology Criteria for Adverse Events
CTFG	Clinical Trial Facilitation Group
CYP	Cytochrome P450
D	Day
DMVs	Double-membrane Vesicle(s)
dsRNA	Double-stranded Ribonucleic Acid
EC	Ethics Committee
ECG	Electrocardiogram
ECMO	Extracorporeal Membrane Oxygenation
e-CRF	Electronic Case Report Form
eEF1A	Eukaryotic Elongation Factor 1A
e.g.	For example, from the latin *exempli gratia*
EOS	End of Study
ET	Early Termination
Etc.	Etcetera
EUA	Emergency Use Authorisation
F	Female
FAS	Full Analysis Set
FDA	U.S. Food and Drug Administration
FiO_2	Fraction of Inspired Oxygen
FLIM-FRET	Fluorescence Lifetime Imaging-Fluorescence Resonance Energy Transfer
g	Grams
GCP	Good Clinical Practice
GGT	Gamma-glutamyltransferase
H	Hours
hACE2	Human Angiotensin converting Enzyme 2
HCoV	Human Coronavirus 227E
HCT	Haematopoietic Cell Transplantation
HCV	Hepatitis C Virus
HEK	Human Embryonic Kidney (cell)
HIV	Human Immunodeficiency Virus
HR	Heart Rate
IB	Investigator's Brochure
IC_{50}	Half (50%) Maximal Inhibitory Concentration
IC_{90}	90% Maximal Inhibitory Concentration

ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IDMC	Independent Data Monitoring Committee
i.e.	That is, from latin i*d est*
IEC	Independent Ethics Committees
IL	Interleukin
IMP	Investigational Medicinal Product
IMV	Invasive Mechanical Ventilation
INN	International Nonproprietary Names
INR	International Normalised Ratio
IRB	Institutional Review Board
IRR	Infusion-related Reactions
IUD	Intrauterine Device
IUS	Intrauterine Hormone-releasing System
ISARIC-4C	International Severe Acute Respiratory and Emerging Infection Consortium-Coronavirus Clinical Characterisation Consortium
ITT	Intention-to-treat
IUPAC	International Union of Pure and Applied Chemistry
IV	Intravenous
K18	Human Keratin 18
L	Liters
LBR	Lung-to-Blood Ratio
LDH	Lactate Dehydrogenase
LLN	Lower Limit of Normal
Log	Logarithm
LoQ	Limit of Quantification
LVEF	Left Ventricular Ejection Fraction
MAbs	Monoclonal Antibodies
MCMC	Markov Chain Monte Carlo
MedDRA	Medical Dictionary for Regulatory Activities
M	Meter
M	Male
mg	Milligram
Min	Minutes
mL	Milliliter(s)

µL	Microliters
mm	Millimeters
MM	Multiple Myeloma
mmHg	Millimeter(s) of Mercury
N	Nucleocapsid/Number
NCI	National Cancer Institute
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NIV	Non-invasive Ventilation
nM	Nanomolar
NT-pro-BNP	N-terminal Pro-B-type Natriuretic Peptide
NYHA	New York Heart Association
PaO_2	Partial Pressure of Oxygen
PaO_2/FiO_2	Partial Pressure of Oxygen Fraction of Inspired Oxygen
Pbar	Barometric Pressure
P-gp	P-glycoprotein
PhV	Pharmacovigilance
PI	Principal Investigator
PK	Pharmacokinetic(s)
PK/PD	Pharmacokinetic/Pharmacodynamic
PO	Oral Administration
PO_2	Partial Pressure of Oxygen
POC	Proof of Concept
PR	Interval PR
q4wk	Every four Weeks
Q4	Quarter 4
qPCR	Quantitative Polymerase Chain Reaction
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
QRS	Interval QRS
QT	QT interval
QTcF	QT Interval Corrected using Fredericia's Formula
qw	Every Week
RAT	Rapid Antigen Test
RBC	Red Blood Cell
RNA	Ribonucleic Acid
RT-PCR	Real Time Polymerase Chain Reaction
SAE(s)	Serious Adverse Event(s)

SAP	Statistical Analysis Plan
SARs	Serious Adverse Reaction(s)
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SBP	Systolic Blood Pressure
SOT	Solid Organ Transplantation
SmPC	Summary of Product Characteristics
SpO2	Saturation of Oxygen
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-emergent Adverse Event
TNFα	Tumour Necrosis Factor alpha
ULN	Upper Limit of Normal
vs	*versus*
WBC	White Blood Cells
WHO	World Health Organization
Wk	Week(s)
WMA	World Medical Association
WOCBP	Women Of Childbearing Potential
wt	Wild-type
Y	Yes

1. INTRODUCTION

1.1 BACKGROUND

Coronavirus disease 2019 (COVID-19), produced by the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), has caused a global pandemic that was initially reported in China in December 2019 (1-3). The clinical manifestations of COVID-19 range from an asymptomatic course to mild (low-level fever, fatigue, and dry cough along with symptoms such as nasal congestion, a runny nose, diarrhea and slight weakness with or without pneumonia) to the severe form, characterized by dyspnoea and/or hypoxemia that can rapidly progress to septic shock, irreversible metabolic acidosis, coagulation disorders, hospital admission, need for intensive care and death in 4.3-15% of patients (3-6). According to the World Health Organization (WHO), as of June 2022, more than 535 million cases of COVID-19 have been reported in the world with more than 6.3 million deaths (7).

Immunocompromised patients are at increased risk for severe and fatal coronavirus disease 2019 (COVID-19) outcomes. In a multicentre retrospective study of over 400 solid organ transplant (SOT) recipients with COVID-19, 78% required hospitalisation, 34% required intensive care, and 27% required mechanical ventilation (8). Haematopoietic cell transplant (HCT) recipients also experience high rates of COVID-19–related complications, with up to 15% of patients requiring intubation (9). Additionally, some studies have shown that mortality rates of SOT and HCT recipients with COVID-19 range between 20% and 30% (8, 9), although more recent studies of SOT recipients, including a study showing comparable outcomes between SOT recipients and non-SOT controls (10, 11), have shown lower mortality rates (4.4%–9.6%) (12, 13). Patients with solid tumors, human immunodeficiency virus (HIV), and primary immunodeficiencies and COVID-19 are also at high risk for severe outcomes, intubation, and death (12, 13). Multiple studies have also demonstrated that vulnerable populations such as patients at increased age with comorbidities are at increased risk of severe COVID- 19 disease and death (13-18). However, data on the outcome of patients with haematological malignancies, especially in the field of malignant lymphoma in patients with COVID-19, are still limited. Lymphoma patients, who are often already immunosuppresed by their disease *per se*, frequently receive lymphodepleting therapies including anti-CD20 monoclonal antibodies, chemotherapy, and other targeted agents also leading to an additional immunosuppressive effect (19). The speed and degree of humoral and cellular immune recovery following prolonged immunosuppression after therapy interruption is also unclear. Futhermore, the impact of different therapeutic approaches in patients with COVID-19 remains poorly defined, but active chemotherapy appears to be associated with increased risk of death in cancer patients with COVID-19 (20). A paradigmatic frame of the high deterioration and mortality risk is sustained by a recent retrospective, multicentre, international study of the data from 63 mantle cell

lymphoma (MCL) patients with a median age of 64 years (44-84) with evidence of a COVID-19 infection. The analysis demonstrated a frequent fatal outcome (44.4%), especially in patients with need for hospitalisation (61%) or intensive care (94%) and undergoing current or recent rituximab therapy (21). The negative impact of SARS-CoV-2 infection in patients with haematological malignancies is also illustrated by a UK-wide retrospective study in relapsed/refractory myeloma (RRMM) patients (n=107) undergoing anti-CD38-based therapy that showed a 159-days cumulative duration of infection-related hospitalisation (22). In line with this, a recent analysis of a retrospective observational study on 593 patients with haematological malignancies included in the EPICOVIDEHA registry suggest that infection with SARS-CoV-2 Omicron variants was associated with considerable morbidity and mortality (23). In fact, the mortality among hospitalised patients with haematological malignancies was found to be 16.5% (23) which is lower than during the COVID-19 waves of 2020 and 2021, but considerable higher than previously reported mortality rates in immunocompetent patients with omicron infections (24-26), and in agreement with a small recent preliminary report on omicron in chronic lymphoid leukemia patients, where 23% 30-day mortality was reported (27). Furthermore, data on serologic response in patients with haematologic malignancies following COVID-19 vaccination are limited. Recent systematic reviews found a correlation between active cancer treatment, particularly anti-CD20 therapy, and lower vaccination response (28, 29). Interestingly, lymphocytopenia, which has been also related with a poor vaccine response, was also associated with progression to critical infection (30). Finally, among patients that progressed to critical infection, vaccination does not seem to have protective effect against death, contrary to treatment with monoclonal antibodies with *in vitro* effect against SARS-CoV-2 Omicron variants (23, 31). These findings underline the need for effective anti-SARS-CoV-2 treatments in these vulnerable cohorts.

Of additional concern is the widespread use of immunosuppressive medications to treat COVID-19 (32). Indeed, the publication of the RECOVERY trial, which showed a significant reduction in mortality among patients with COVID-19 who received dexamethasone (33), resulted in a paradigm shift in the medical management of COVID-19, whereby immunomodulation and not antiviral therapy has become an accepted clinical practice standard. Therefore, current guidelines endorse the use of dexamethasone and IL-6 inhibitor therapy in subgroups of patients with COVID-19 without excluding immunocompromised patients (34), even though these patients were generally excluded from clinical trials. Moreover, given that immunosuppressed patients are at risk for protracted SARS-CoV-2 infection, concerns have been raised about whether immunosuppressive therapies may promote chronic infection and worse outcomes, including selection and transmission of new SARS-CoV-2 variants (32). Thus, studies among immunocompromised hosts should ideally be focused on identifying direct-acting antivirals that markedly reduce SARS-CoV-2 replication, be they small molecules

or antibodies. So far, however, patients with immune-system disorders have not been sufficiently represented in the clinical trials for COVID-19 antiviral drugs. At the appendix "Material" of ACCT-1 trial of remdesivir, immune-depressed patients are only a 7% of patients of the 1,062 patients included (32 in the remdesivir arm and 41 in the placebo arm) (35). The number is almost the same for patients with neoplasm malignant with 43 patients included in the remdesivir arm and 37 in the placebo arm representing 8% of the study population (35). Throughout the study analysis, patients with immune-system disorders were grouped with patients with other more frequent comorbidities such as hypertension and obesity and no subgroup analysis was performed (35). A more recent trial, published in 2022 (remdesivir plus dexamethasone *versus* dexamethasone alone also did not have a subgroup of immune-system disorders as comorbidities described (36). So, although the efficacy of remdesivir has been questioned due to conflicting data (35, 37), this RNA polymerase inhibitor and other antivirals such as molnupiravir (NCT04405739) (38) should also be studied in clinical trials of immunocompromised hosts who may reap the greatest benefit from such interventions. Regarding the use of monoclonal antibodies, which are already available for administration to both immunocompromised and immunocompetent patients in the United States via an emergency use authorization (EUA), they should now be expanded via clinical trials to encompass settings beyond those outlined in the EUA (e.g., early hospitalisation, supplement oxygen requirement in patients with prolonged replication), with a focus on immunocompromised patients who may benefit the most from them. It should be pointed out, however, the marked loss of activity of many monoclonal antibodies (MAbs) against SARS-CoV-2 Omicron variants that underscores the importance of developing MAbs that target conserved regions of spike receptor-binding-domain (39).

1.2 INFORMATION ON THE STUDY DRUG

In the set of preclinical studies, plitidepsin showed strong antiviral activity in *in vitro* models of SARS-CoV-2 infection, also showing a better therapeutic index than other drugs, including remdesivir (40, 41). In fact, highly consistent results were obtained, with the IC_{50} of plitidepsin always being in the nanomolar range, regardless of the coronavirus species (HCoV 227E, SARS-CoV, and SARS-CoV-2), the infected host cells, or the quantifying method used (42). Notably, a similar *in vitro* antiviral effect was induced by plitidepsin against the α, β, δ, μ, and o variants of SARS-CoV-2, which are known to bear several mutations affecting the viral spike protein that facilitates viral entry through its interaction with the hACE2 receptor (**Table 1**) (42, 43).

Table 1 Plitidepsin in vitro activity against different SARS-CoV-2 variants.

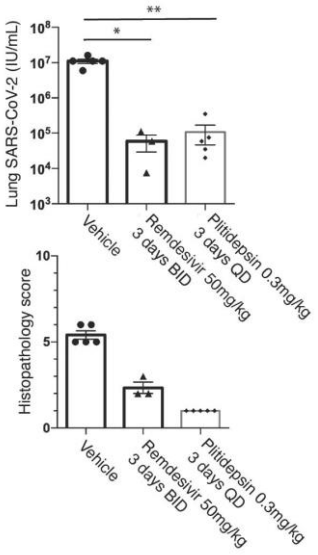
Variant	Strain	IC_{50} (nM)	CC_{50} (nM)	Selectivity Index
Wild type	WA1	0.20	11.1	56

Alpha	B.1.1.7	0.05	11.1	222
Beta	B.1.351	0.08	11.1	139
Delta	B.1.617.2	0.09	11.1	123
Mu	B.1.621	0.27	11.1	41
Omicron	B.1.1.529	0.29	6.3	22

IC_{50}: 50% of maximum SARS-CoV-2 inhibitory capacity; CC_{50}: 50% of maximum cytotoxic effect on host cells (HeLa-ACE2); Selectivity Index: CC_{50}/IC_{50}.

A recent study showed that plitidepsin treatment (at concentrations in nanomolar range) of Vero E6 cells infected with SARS-CoV-2 completely abolished the formation of double-membrane vesicles (DMVs), organelles that support coronavirus genome replication (44). Additionally, neither SARS-CoV-2 nucleocapsid (N) protein nor double-stranded RNA (dsRNA) were present in plitidepsin-treated infected cells (44). Altogether, these results showed that plitidepsin treatment completely blocked the assembly of SARS-CoV-2 viral structures in infected cells.

In addition, White *et al* (2021) also demonstrated a strong antiviral activity *in vivo*, characterised by a significant reduction in the viral load in lungs, as well as a clear reduction in alveolar and peribronchial inflammation (**Figure 1**) (40).



Remdesivir, n=3; plitidepsin 0.3 mg/kg and vehicle, n=5. Taken from (White et al 2021).

*p<0.05, **p<0.01.

Figure 1 Viral load (top) and histopathology score of inflammation (bottom) in the lung of K18-hACE2 mouse model, infected with SARS-CoV-2 and treated with plitidepsin.

1.2.1 Plitidepsin

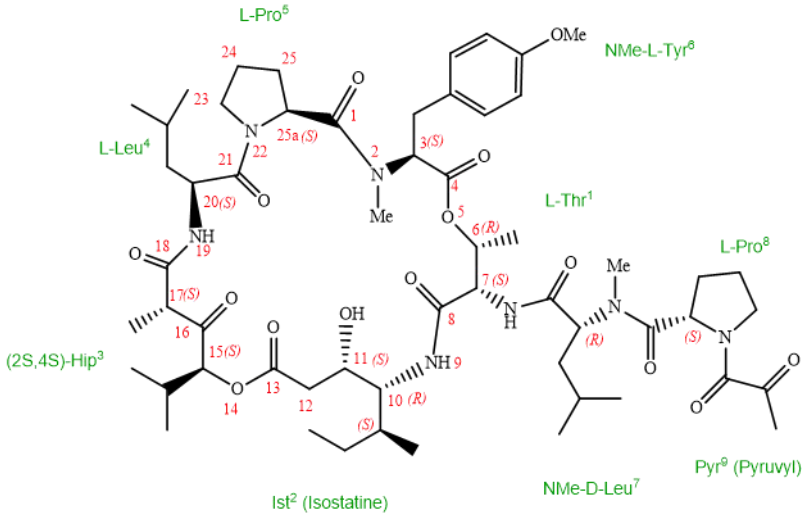
Plitidepsin is a cyclic depsipeptide that was first isolated from a Mediterranean tunicate (*Aplidium Albicans*). Currently plitidepsin is manufactured by synthesis.

Plitidepsin was approved in Australia in 2018 for the treatment of relapsed/refractory multiple myeloma (oncology) and is in development for the treatment of COVID-19.

1.2.1.1 ***Name and Chemical Information***

Plitidepsin is synthesised chemically. The structure is summarised below:

INN Name:	plitidepsin
IUPAC Name:	(-)-(3*S*,6*R*,7*S*,10*R*,11*S*,15*S*,17*S*,20*S*,25a*S*)-11-hydroxy-3-(4-methoxybenzyl)-2,6,17-trimethyl-15-(1-methylethyl)-7-[[(2*R*)-4-methyl-

	2-[methyl[[(2*S*)-1-(2-oxopropanoyl)pyrrolidin-2-yl]carbonyl]amino]pentanoyl]amino]-10-[(1*S*)-1-methylpropyl]-20-(2-methylpropyl)tetradecahydro-15*H*-pyrrolo[2,1-f]-[1,15,4,7,10,20]dioxatetrazacyclotricosine-1,4,8,13,16,18,21(17H)-heptone
Laboratory Names:	aplidin, aplidine, dehydrodidemnin-B, APLD, DDB, PM90001
Molecular Formula:	$C_{57}H_{87}N_7O_{15}$
Molecular Mass:	1110.3 g/mol
Structural Formula:	

1.2.1.2 ***Mechanism of Action***

The target for plitidepsin's anti-tumour and anti-viral activity is eukaryotic elongation factor 1 alpha (eEF1A).

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 (45). 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 (45).

As viruses depend on host cell proteins for their replication and propagation, it is not surprising that they have apparently evolved to utilize eEF1A in their life cycle (46). 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 (47). Coronavirus N protein was shown to interact directly with eEF1A (48) (49) (50) and viral replication was inhibited by eEF1A knockdown (49) or pharmacologic inhibition (50) in host cells, suggesting the interaction between eEF1A and coronavirus N protein is essential.

To determine whether the plitidepsin effect on SARS-CoV-2 virus replication may be explained by inhibition of eEF1A, a set of *in vitro* experiments were performed (40). Briefly, human embryonic kidney 293T (HEK293T) cells were co-transfected with a lentivirus expressing human angiotensin converting enzyme 2 (hACE2) and with different plasmids expressing either EF1A wild type (wt) or EF1A-A399V (which contains an amino acid mutation that induces resistance to plitidepsin structurally related compounds (51)). Each of these transfected HEK293T-hACE2 cell lines was infected with SARS-CoV-2 and the effect of plitidepsin treatment assessed. Results showed an increase of the IC90 of plitidepsin by a factor of >10 against infected cells transfected with EF1A-A399V compared with EF1A-wt. Additionally, EF1A-A339V CRISPR knock-in HEK293T-hACE2 cells infected with SARS-CoV-2 was refractory to the SARS-CoV-2 antiviral activity of plitidepsin by a factor of >12 as compared to the parental cell line. These results confirmed that plitidepsin inhibition of EF1A is the molecular basis for plitidepsin antiviral effects.

1.2.1.3 ***Summary from the Clinical Development of Plitidepsin***

Plitidepsin has been used as an experimental therapy in 947 patients with different types of solid tumors and haematological malignancies, across 24 clinical trials. The clinical pharmacokinetics (PK) of plitidepsin has been well characterised in phase 2/3 studies involving 193 adults with multiple myeloma and phase 1/2 studies involving 509 adults with other types of malignancies (**Table 2**). The administered doses of plitidepsin in these studies ranged from 0.13 to 8.0 mg/m2 as 1-hour, 3-hour, and 24-hour constant rate IV infusions. Please refer to the Investigator Brochure (IB) of plitidepsin for an updated and thorough overview of clinical information.

Table 2 Clinical Studies and Pooled Analysis Supporting the Plitidepsin Clinical Development Program

Study	Population	Doses (mg/m²)	Infusion length & regimen	Patients treated/ with PK	Study completion
Phase 1 Studies In Patients With Solid and Haematological Tumours					
APL-A-001a-98 (52)	Solid tumours, NHL	0.13 to 4.50	24-h D1, 8, 15 q4wk	35/34	September 2002
APL-A-001b-98 (53)	Solid tumours, NHL	3.00 to 6.00	3-h D1, 15 q4wk	27/26	August 2002
APL-A-002-98 (54)	Solid tumours, NHL	0.13 to 3.60	1-h D1, 8, 15 q4wk	49/46	December 2002
APL-A-003-98 (55)	Solid tumours, NHL	0.20 to 8.00	24-h D1, 15 q4wk	67/48	April 2003
APL-A-004-98 (56)	Solid tumours, NHL	0.08 – 1.50	1-h D1-5 q3wk	37/37	May 2002
Phase 1 Combination Studies					
APL-A-006-05 (57)	Solid tumours, lymphoma, with carboplatin	1.80 – 3.00	1-h D1, 8, 15 q4wk	20/20	September 2007
APL-A-010-08[a]	Solid tumours, lymphomas, with gemcitabine or sorafenib	1.80 – 3.00	1-h D1, 8, 15 q4wk	44/32	December 2011
APL-A-011-08 (58)	Solid tumours, with docetaxel or bevacizumab	2.80 – 4.80	3-h D1, 15 q4wk	18/13	July 2010
APL-A-012-13 (59)	MM, with bortezomib	4.00 – 5.00	3-h D1, 15 q4wk	22/17	April 2016
Phase 2 Studies in Patients with Solid Tumours					
APL-B-001-01 (60)	Renal cancer, colorectal cancer	5.00 to 7.00	24-h D1, 15 q4wk	81/43	February 2006
APL-B-002-02[c] **(61)**	Medullary thyroid carcinoma	4.25 to 5.00	3-h D1, 15 q4wk	16/16	February 2006
APL-B-003-02[a,c]	Pancreatic cancer	5.00	3-h D1, 15 q4wk	19/-	March 2006
APL-B-004-02[c] (62)	Non-small cell lung cancer	5.00	3-h D1, 15 q4wk	21/-	January 2005
APL-B-005-02[c] **(63)**	Carcinoma of the urothelium	5.00	3-h D1, 15 q4wk	21/20	February 2007
APL-B-006-02[c(64)]	Small-cell lung cancer	2.00 to 3.20	1-h D1, 8, 15 q4wk	19/15	May 2006
APL-B-007-02[c] **(65)**	Melanoma	5.00	3-h D1, 15 q4wk	37/37	September 2005
APL-B-010-02[a,c]	Head and neck cancer	5.00	3-h D1, 15 q4wk	10/-	March 2005
APL-B-011-02[a,c]	Prostate cancer	5.00	3-h D1, 15 q4wk	8/8	December 2007
APL-B-016-05[c] **(66)**	Melanoma, with or without dacarbazine	1.80 – 3.20	1-h D1, 8, 15 q4wk	84/67	October 2010
Phase 2 and phase 3 Studies in Patients with Haematological Tumours					
APL-B-013-02[c] **(67)**	NHL	3.20	1-h D1, 8, 15 q4wk	34/24	June 2010
APL-B-014-03[c] **(68)**	MM, with or without DXM	5.00	3-h D1, 15 q4wk	51/45	June 2008
APL-B-015-04[a]	ALL	3.20	1-h D1, 8, 15 q4wk	17/17	July 2006
APL-C-001-09[c] **(69)**	MM, with DXM	5.00	3-h D1, 15 q4wk	204/139	November 2015
Human Mass Balance Study					
APL-A-013-13 (70)	Solid tumours	7.00 mg FD	3-h D1, 15 q4wk	6/6	January 2016

Study	Population	Doses (mg/m²)	Infusion length & regimen	Patients treated/ with PK	Study completion
Phase 1 Study in Patients with COVID-19					
APLICOV-PC (42)	COVID-19	1.5 to 2.5 mg FD	1.5-h D1-3	45/-	Nov 2020

[a] Ongoing clinical trials or unpublished results.

[b] Patients with PK pooled from phase 1-3 studies listed above.

[c] Studies included in the integrated analysis of AEs after the first infusion of treatment (cycle 1/Day 1)

ALL: acute lymphoblastic leukaemia; D: day; DXM: dexamethasone; FD: flat dose; h: hour; m^2: square metre; mg: milligram; MM: multiple myeloma; NA: not applicable; NHL: non-Hodgkin's lymphoma; PK: pharmacokinetic; q: every; wk: week

As shown in the above **Table 2** (APL-A-004-98 study), the multi-day 60-min IV infusion schedule of plitidepsin was initially explored in a phase 1 trial in oncology (56). The Recommended Dose for Phase 2 trials was defined as 1.2 mg/m^2/day for 5 consecutive days, and was found to be safe in seven patients from this study who received it. These patients received an actual median daily dose of 2.2 mg (range 1.9 – 2.7 mg/day), corresponding to a total median dose of 11.2 mg over a 5-day period (range: 9.6 – 13.7 mg).

APLICOV-PC was the first trial developed in COVID-19 (42). It explored three doses of plitidepsin, delivered daily over 3 consecutive days: 1.5, 2 and 2.5 mg/day. The three doses showed acceptable safety for further development in COVID-19 (See IB v5.0, section 5.3). NEPTUNO is the phase III trial in hospitalised patients with moderate COVID-19 requiring oxygen supplementation. It explores the efficacy and safety of two doses of plitidepsin (1.5 and 2.5 mg/day) *versus* a control group. It has recently been closed for accrual and results are not available yet. Nevertheless, a futility safety analysis performed when there were at least 30 patients per arm with 30-days follow-up did not find any alert signal.

Regarding the infusion time selection, the clinical development of plitidepsin as an anticancer compound (summarized in the above **Table 2**) initially explored three different lengths of infusion, 1-h, 3-h and 24-h. APLICOV-PC, is the only study conducted with plitidepsin that has used a 90-min IV daily infusion in a total of 45 patients. The rationale for the daily administration schedule of APLICOV-PC was discussed with the Spanish Medicines Agency and stemmed from a conservative extrapolation from the results of a phase 1 trial performed in cancer patients (56). The 60-minute IV infusion schedule was the one selected for the NEPTUNO trial. The population PK model that supported the selected doses of plitidepsin in COVID-19 was used to compare the simulated plasma PK profiles when plitidepsin is administered as 1 h and 1.5 h infusion (see **Figure 2**). The expected differences in Cmax between both infusion lengths are small. Moreover, Cmax with the 2.5 mg dose given as a 1-h infusion is expected to be considerably below median Cmax values achieved in more than 300 patients with different tumor types (**Figure 3**), for whom no safety signals of concern were observed after the first infusion of plitidepsin (IB section 5.2.2).

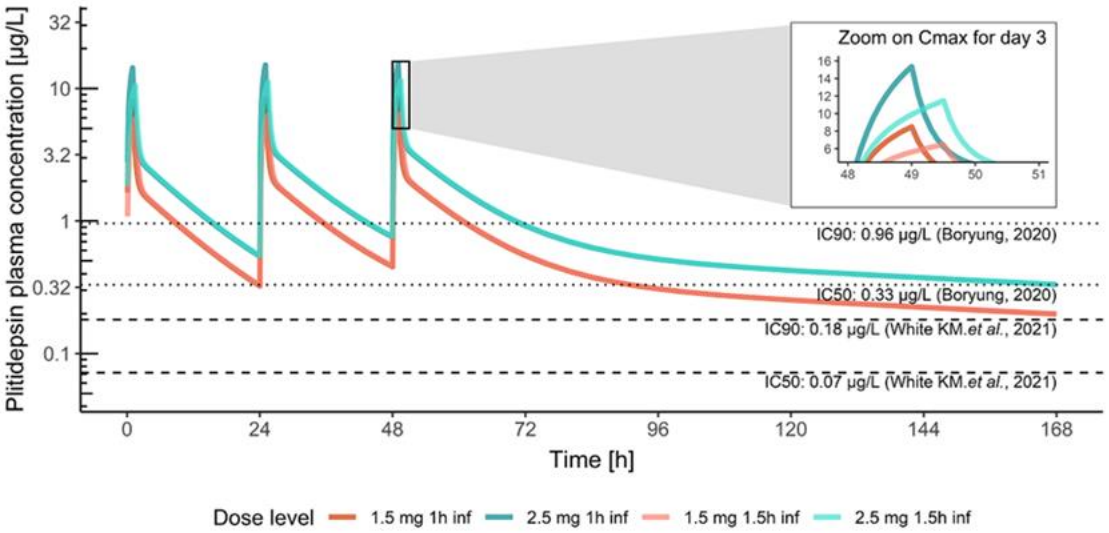


Figure 1 Total plasma concentration profiles vs plitidepsin time predicted between 1 hour and 1.5 hour.

In NEPTUNO Phase III trial, a futility analysis was performed with at least 30 patients per arm and 30-days follow-up. No alert signals were found, as concluded by the IDMC, reinforcing the safety of the 60-min IV infusion scheme previously assayed the trials displayed in **Table 2**.

The IB tabulates the safety of multiple treatments of plitidepsin delivered weekly at 60-min IV infusion, at the recommended safe dose of 3.2 mg/m^2 (IB: Table 7 – group C), higher than the daily flat dose of 2.5 mg.

In parallel, the compassionate use of plitidepsin was granted to treat immune-compromised patients with COVID-19, not eligible for NEPTUNO trial. This experience has been compiled by the treating physicians as a retrospective analysis on 33 patients (71). The majority of the patients received the dose of 2.5 mg/day, except for 4 frail patients with organ dysfunction, who received 1.5 mg. A total of 111 doses of plitidepsin have been delivered as 60-min IV infusions, with a lack of infusion-related and serious systemic adverse events. With the caveat of being a retrospective study, this information is important as mimics the target population expected to be included into NEREIDA trial.

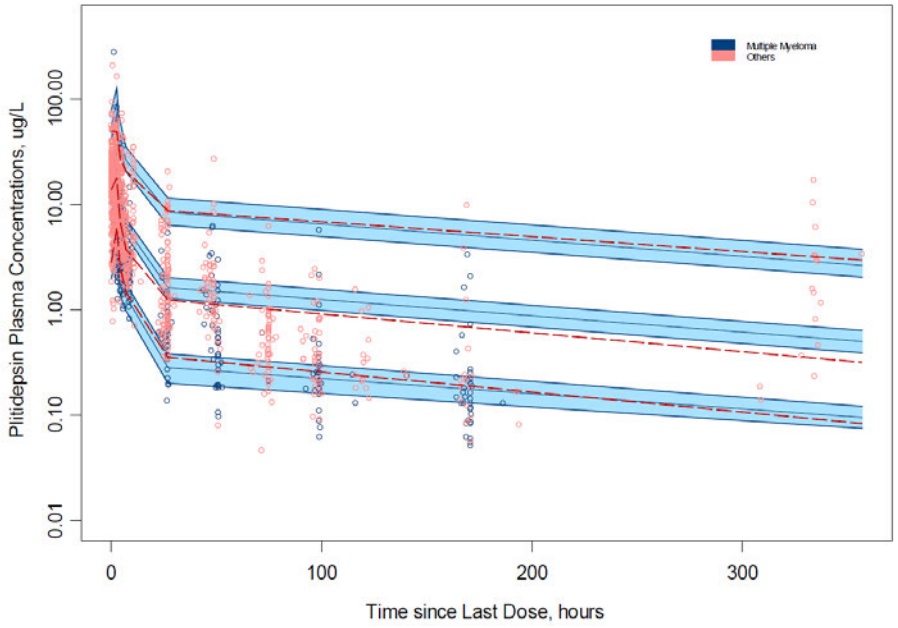


Figure 2 Visual predictive check describing the time course of plitidepsin plasma in different tumor types. Blue lines and areas represent the median and 95% confidence intervals of the 5, 50 and 95th percentiles of the model-based predictions, and dots represents the plasma plitidepsin concentrations.

1.2.1.3.1 Benefit-Risk Considerations in COVID-19

Plitidepsin has also been evaluated in a proof of concept (POC) study (APLICOV-PC) that included 46 hospitalised COVID-19 patients enrolled across 10 sites in Spain. As mentioned above, three doses of plitidepsin delivered as a 90-minute IV infusion were studied in this trial: 1.5, 2, and 2.5 mg, daily for 3 consecutive days (corresponding to 4.5, 6, and 7.5 mg per single course). Safety was the primary outcome of the POC study, which was assessed by the frequency of grade ≥ 3 AEs based on NCI CTCAE v.5.0 criteria, the percentage of patients with treatment-related and unrelated AEs, serious adverse events (SAEs), changes from baseline in haematologic and non-haematologic parameters, and percentages of patients with electrocardiogram (ECG) abnormalities (42).

AEs regardless of relationship are summarised by dose cohort and overall in Table 17 of the IB. Overall, 98% (44/45) of patients experienced at least one AE. The most common AEs by organ system, occurring in 2 or more (≥4.4%) of patients overall were:

- Haematologic disorders: leukocytosis (6.7%), lymphopenia (6.7%)
- Gastrointestinal disorders: abdominal pain (8.9%), constipation (17.8%), diarrhoea (17.8%), dyspepsia (8.9%), nausea (42.2%), vomiting (17.8%)
- General disorders: asthenia (13.3%), pyrexia (44.4%)

- Infections: bacteremia (4.4%), COVID-19 pneumonia (6.7%), pneumonia (4.4%), urinary tract infection (4.4%)
- Investigations: ALT increased (11.1%), AST increased (6.7%), alkaline phosphatase increased (4.4%), C-reactive protein increased (24.4%), CPK increased (4.4%), ferritin increased (31.1%), fibrin D-dimer increased (17.8%), GGT increased (13.3%), LDH increased (8.9%), triglycerides increased (4.4%)
- Metabolic and nutrition disorders: decreased appetite (4.4%), hyperglycaemia (17.8%)
- Musculoskeletal disorders: back pain (4.4%)
- Nervous system disorders: dizziness (6.7%), dysgeusia (4.4%), headache (13.3%), insomnia (8.9%), presyncope (8.9%), somnolence (4.4%)
- Psychiatric disorders: delirium (4.4%)
- Renal: pre-renal failure (4.4%), renal impairment (4.4%)
- Respiratory: acute respiratory distress syndrome (8.9%), acute respiratory failure (4.4%), cough (24.4%), dyspnoea (15.6%), hypoxia (4.4%), tachypnea (4.4%)
- Vascular disorders: hypertension (4.4%), phlebitis (17.8%)

No significant effects were observed on atrioventricular conduction or on depolarization in the protocol-specified analysis of ECGs, as no patient showed corrected QT interval by Fredericia [QTcF] >480 ms or change from baseline in QTcF >60 ms. Additionally, no significant effects were observed on atrioventricular conduction or on depolarisation, as measured by mean changes in PR and QRS intervals.

Liver test abnormalities: Although the majority of patients in the APLICOV-PC study experienced new or worsening abnormal liver function tests on study (with lab tests graded according to CTCAE v5.0), including increased ALT (65.9%), increased AST (29.5%), and increased GGT (38.6%), these events were reversible, mild, and short-lasting, as shown in Figure 10 of the IB for ALT, and no cases of hepatocellular injury were reported in the APLICOV-PC study. Notably, about one-third of patients had elevated liver function tests at baseline, including elevated ALT (grade 1 in 34.1% of patients), elevated AST (grade 1 in 36.4%), elevated GGT (grade 1 in 36.4%, grade 2 in 6.8% and grade 3 in 2.3%) and elevated LDH (grade 1 in 52.3%), but none had elevated bilirubin at baseline and no patients reported liver disease comorbidities at baseline. Although other studies of hospitalised COVID-19 patients have reported increased abnormal liver tests and increased liver injury with the use of the antiviral agents lopinavir, ritonavir and remdesivir (72, 73), there was no evidence of plitidepsin-induced liver injury in the APLICOV-PC study.

Nausea and vomiting: Nausea and vomiting were the most commonly reported AEs. A protocol amendment "AR9" was implemented in August 2020 to modify the prophylactic premedication given prior to plitidepsin infusion. Ondansetron 8 mg IV slow infusion was added and route of administration of dexamethasone was changed from oral to IV. The implementation of the aforementioned protocol amendment was associated with a reduction in the proportion of patients with nausea (from 55.5% to 38.9%) and vomiting (from 22.2% to 13.9%) and no new hypersensitivity reactions were seen in any of the 36 patients treated after the amendment (108 infusions) (See Table 18 of the IB).

Treatment was well tolerated, with equivalent safety outcomes in all three dose cohorts (42). Only 31% (14/45) of the patients experienced Grade ≥3 AEs and only 2 patients experienced a treatment-related Grade ≥3 AE: one case of anaphylactic reaction within 5 minutes of starting the first plitidepsin infusion that resolved with pharmacologic treatment but resulted in treatment discontinuation and one case of diarrhoea that had no impact on study treatment (42).

Considering the low rate of drug-related Grade ≥3 AEs and the high discharge rate at Day 15 (82% in the full study population; 100%, 96%, and 53% in patients with FDA mild, moderate, and severe disease at baseline, respectively), along with an average 3.25-$\log_{10}$ reduction in baseline viral load by Day 15, evidence from the APLICOV-PC study indicated a positive benefit-risk for plitidepsin for treatment of patients hospitalised for COVID-19 and justified initiation of the randomised, controlled NEPTUNO trial to establish tolerance and efficacy. This study is blinded to the Sponsor for analysis, and therefore no clinical information can currently be given.

1.2.1.3.2 Effects of Plitidepsin in Immune System

An integrated analysis on the safety of the recommended doses for Phase 2-3 clinical trials have been done on a total of 419 cancer patients after their first infusion of treatment (cycle 1/Day 1) (Please refer to the IB of plitidepsin). This analysis also evaluated the potential immunosuppressive effects of plitidepsin based on the following parameters:

- Incidence of infections: The most significant side effect of immunosuppressive drugs is an increased risk of infection. Among the 419 cancer patients, 47 (11.21%) patients experienced 55 infections after their first infusion of treatment (cycle 1/Day 1), none of which were considered by Investigators to be related to study drug. A total of 21 infections in 19 (4.5%) patients were reported as SAEs, resulting in prolonged hospitalisation for 18 patients and death of one patient as a result of a pneumococcal infection and sepsis. Among SAEs for infection, those reported in more than one patient were pneumonia (7 patients), respiratory tract infection (3), sepsis (2) and infection (2).
- Incidence of lymphopenia and neutropenia: Lymphopenia and neutropenia increase susceptibility to infection. Among the 419 cancer patients, there were no reports of lymphopenia and reports of neutropenia (grade 3) for only 3 (0.7%) patients after the first infusion of treatment (cycle 1/Day 1). The low incidence of neutropenia and absence of lymphopenia support Investigator assessments that AEs of infections were unrelated to plitidepsin treatment.
- Incidence of laboratory abnormalities in lymphocytes and neutrophils: Shift tables for the change from baseline to the worst grade on study for lymphocyte count and neutrophil count showed new or worsening decreases in lymphocyte count for 15.5% (61/394) of evaluable patients and new or worsening decreases in neutrophil count for 9.9% (24/396) of evaluable patients. Most patients (75.9% [299/394]) had grade 0-1 lymphocyte count at baseline and only 4 (1.3%) of these patients developed grade 3 lymphocyte count decrease on study.

Similarly, most patients (85.9% [340/396]) had grade 0-1 neutrophil count at baseline and only 1 (0.3%) developed grade 3 neutrophil count decrease on study. The variation of lymphocytes counts and neutrophil counts after the first infusion of treatment (cycle 1/Day 1) is presented in **Figure 4** and **Figure 5**, respectively.

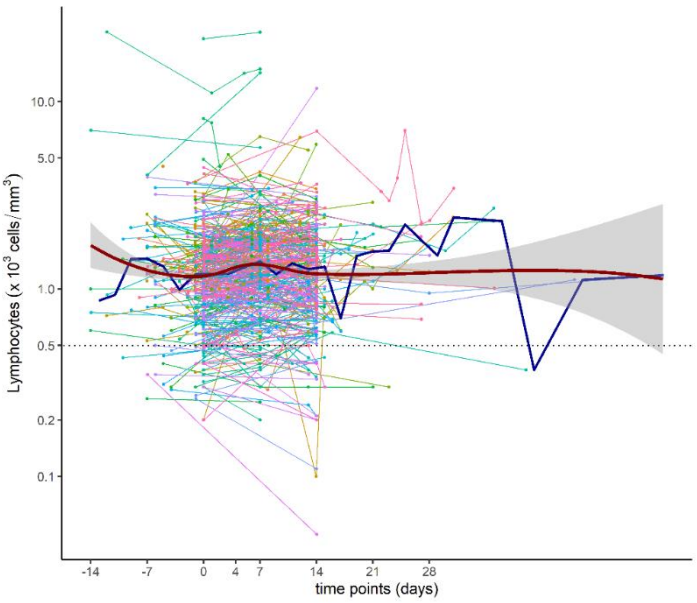


Figure 4 Time variation of lymphocyte counts after the first infusion of treatment (cycle 1/Day 1).

Each line represents the analytical variation of a single patient. Bold blue line connects the median values obtained at bins of 2 consecutive time points. Bold red line represents a LOESS polynomic regression. Shaded area plots 95% confidence limits.

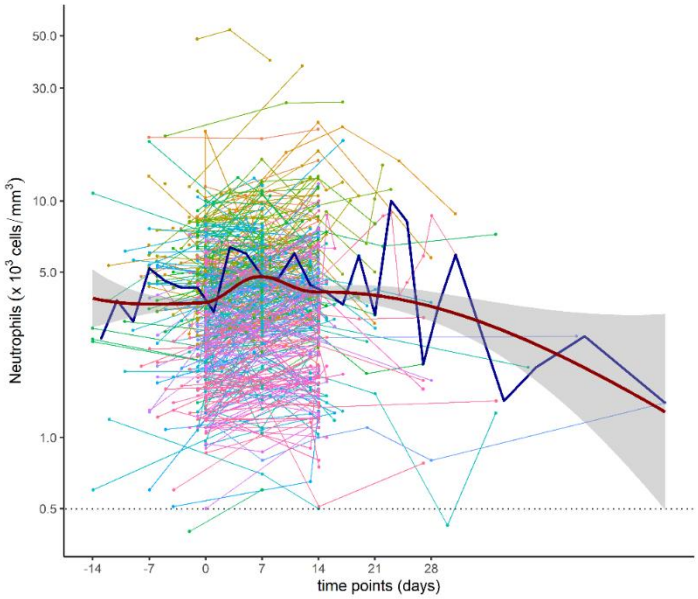


Figure 5 Time variation of neutrophil counts after the first infusion of treatment (cycle 1/Day1).

Each line represents the analytical variation of a single patient. Bold blue line connects the median values obtained at bins of 2 consecutive time points. Bold red line represents a LOESS polynomic regression. Shaded area plots 95% confidence limits.

In the same way, the potential immunosuppressive effects of plitidepsin were studied in patients with COVID-19 participating in APLICOV-PC, by examining:

- Incidence of concomitant infections: The most significant side effect of immunosuppressive drugs is increased risk of infection. Among the 45 patients hospitalised for management of COVID-19 in the APLICOV-PC study, only 10 AEs for infections other than COVID-19 were reported, none of which were considered by Investigators to be related to plitidepsin treatment.
- Incidence of lymphopenia and neutropenia: Lymphopenia and neutropenia increase susceptibility to infection. Among the 45 COVID-19 patients, no AE of neutropenia and only 3 AEs of lymphopenia were reported, none of which were considered by Investigators to be related to plitidepsin treatment.

  Although several studies have examined the causes of lymphopenia during viral infections, the mechanisms underlying lymphopenia in COVID-19 patients is not fully understood. Possible underlying causes include redistribution of T cells into infected organs, activation induced exhaustion, apoptosis, or pyroptosis (74, 75)
- Incidence of laboratory abnormalities in lymphocytes and neutrophils: At baseline, 52.3% of patients had reduced absolute lymphocyte count (grade 1 in 47.7% and grade 2 in 4.5%) and while on study 31.8% of patients developed new or worsening lymphopenia, with acute onset (**Figure 6**). However, no events of new or worsening lymphopenia were considered by

Investigators to be related to plitidepsin treatment, as low lymphocyte count (lymphopenia) is one of the clinical indicators of the severity of SARS-CoV-2 infection (76, 77).

At baseline, all patients had normal neutrophil counts and while on study only one patient developed grade 1 neutropenia.

An isolated observation of Grade 3 neutropenia was reported in an asymptomatic outpatient on the Day 31 follow-up; this patient was also taking metamizole, and the responsible investigator deemed that the event was neither clinically relevant nor related to plitidepsin.

Notably, despite the fact that 64.4% of patients received dexamethasone for more than 3 days, they exhibited a median increase in the absolute number of lymphocytes (**Figure 6**).

The lack of a control group prevents conclusive evidence, but this could be a very interesting finding given that, in addition to glucocorticoid therapy, SARS-CoV-2 infection can also induce an early functional exhaustion of cytotoxic lymphocytes that may be responsible for delaying immune responses (78, 79).

- Prolonged viral replication of SARS-CoV-2: recent publications report prolonged viral replication of SARS-CoV-2 in immunocompromised patients (80-82). As discussed in efficacy results, the -3.25 $\log_{10}$ mean reduction from baseline viral load at Day 15 in the APLICOV-PC study support the hypothesis that plitidepsin has no immunosuppressive effects, as viral replication is shortened, not prolonged with plitidepsin treatment.

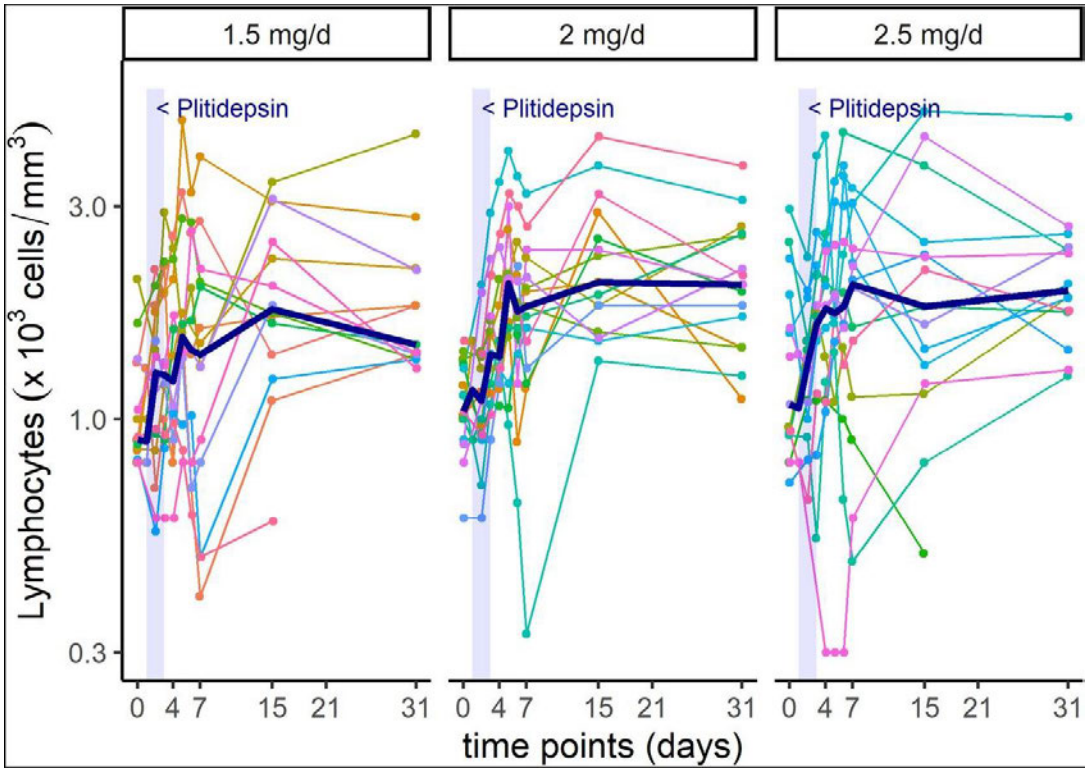


Figure 6 Intra-patient Time Variation in Lymphocyte Count per Dose Cohort and Median Trends.

Source: Appendix 16.1.9 – Figure 15.3.13.

Each colour represents a patient and the bold dark blue line is the median trend, ULN: upper limit of normal

1.2.1.3.3 Clinical data of plitidepsin in immunocompromised patients with COVID-19

Recently published data on the compassionate use of plitidepsin in cancer and immunocompromised patients with COVID-19 reinforce the safety of the drug (71, 83, 84).

The first case report described the use of plitidepsin for management of a cancer patient infected with COVID-19 while receiving chemotherapy (83). The investigator noted that patients with cancer who also are infected with COVID-19 have a poor prognosis and increased risk of all-cause mortality. Additionally, cancer treatments are almost always withheld from patients with COVID-19, leading to an increased risk of tumor-related morbidities. In this case, a 75-year old male with stage IIIB gastric signet ring cell carcinoma was diagnosed with COVID-19 shortly after receiving his first course of FOLFOX-4 chemotherapy. After his COVID-19 diagnosis, he received two courses of levofloxacin and dexamethasone over 10 days. After 22 days of continued illness, he was hospitalised and treated with dexamethasone combined with

cyclosporine A without any improvement in his signs of respiratory failure; his lymphopenia remained unchanged and he continued to test positive for SARS-CoV-2 by RT-PCR. He was then given one full course of plitidepsin (2.5 mg once daily for 3 days). He showed a substantial acute reduction in viral load 4 and 7 days after initiating plitidepsin treatment (**Figure 7**). The patient was discharged 18 days after plitidepsin treatment, having received a full second course of chemotherapy with only a one-week delay from the planned schedule. All three cycles of FOLFOX-4 were well tolerated by the patient, with no signs of bone marrow or organ toxicities and plitidepsin treatment did not induce any safety signals that would interfere with anticancer therapy.

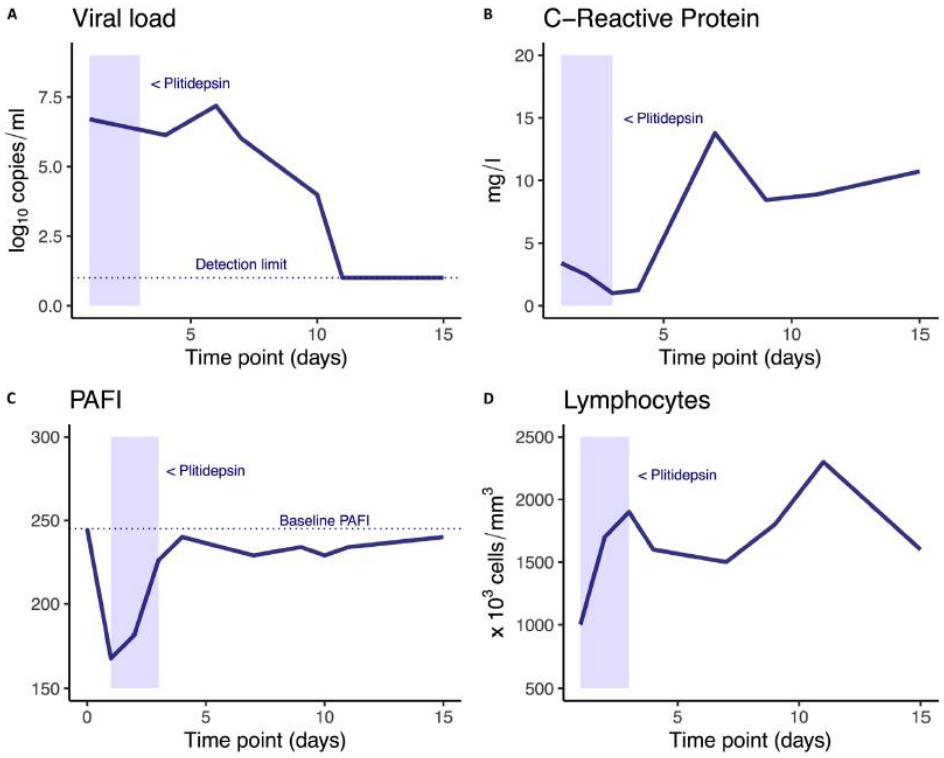


Figure 7 Timeline of principle laboratory and microbiological parameters taking Day 1 of plitidepsin therapy as reference, and during hospital admission.

(A) Quantitative viral load (log10 copies/ml) using nasopharyngeal swabs samples. (B) Plasma C-reactive protein (mg/l) levels. (C) PAFI, or PaO2/FiO2 ratio in mmHg. (D) Lymphocytes total count (x10^3 cells mm^3) during and after plitidepsin therapy. PAFI, a ratio of the partial pressure of oxygen (PaO2) to a fraction of inspired oxygen (FiO2) (PaO2/FiO2) (83).

The second case report described the use of plitidepsin for management of a cancer patient infected with COVID-19 who had previous anti-CD20 monoclonal antibody-mediated B cell depletion and chronic lymphocytic leukemia (CLL) (84). In this case, a 75-year old male with a previous history of CLL was diagnosed with COVID-19 after a week of dry cough and tiredness.

After 22 days of continued illness, he was hospitalised for persistent fever, fatigue, the appearance of respiratory insufficiency and ongoing bilateral opacities by chest X-ray; the patient had undetectable levels of antibodies against SARS-CoV-2 and had undetectable levels of CD19+ and CD20+ B cells in peripheral blood (due to his prior treatment for CLL). The patient experienced further clinical and image severity progression and a course of plitidepsin (2.5 mg once daily for three days) was initiated 48 days after onset of COVID-19. Clinical improvement was apparent shortly after plitidepsin treatment, which led to withdrawal of oxygen therapy 7 days after the first dose of plitidepsin. On Day 61 after onset of COVID-19, the patient was given an IV infusion of human immunoglobulin, followed 24 hours later by a second course of plitidepsin. On Day 74 the patient received his first negative RT-PCR test and was subsequently negative for SARS-CoV-2 in three consecutive samples (**Figure 8**). The patient was discharged from hospital on Day 82 and did not experience any signs of COVID-19 relapse during six months follow-up.

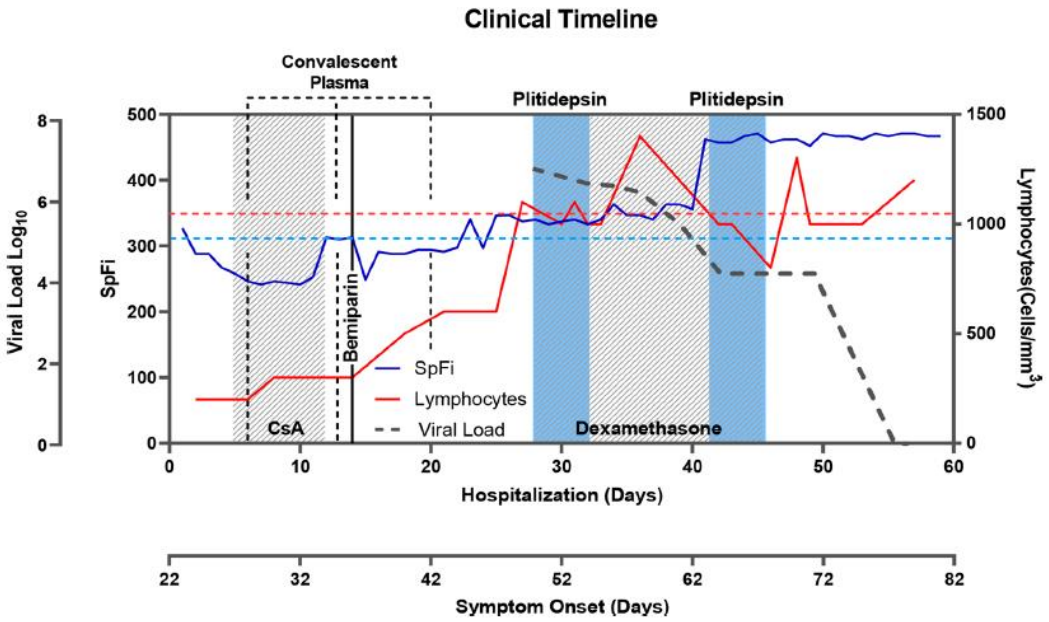


Figure 8 Timeline of main laboratory and microbiological parameters during and after plitidepsin therapy.

Two timepoints have been taken as reference: the number of days from SARS-CoV-2 symptom onset, and the number of days since hospital admission. Plitidepsin was administered on Days 49–51 and 65–67 after symptom onset. Parameters shown in the clinical course are as follows: A Quantitative viral load (log10 copies/ml) using nasopharyngeal swabs samples (grey-dotted line). B SpFI (AU) blue line. C Lymphocyte total count (cells/mm3) (red line). *AU* arbitrary units, *CsA* cyclosporine A, *SpFi* ratio of oxygen saturation in blood (SpO2)/fraction of inspired oxygen (FiO2) at or below 300 AU (84).

A small observational retrospective study on immunocompromised patients (n=33) treated with at least one full course of plitidepsin under compassionate use for the management of COVID-19 is also being carried out as an investigator-sponsored study (see also Section 1.2.1.3

Summary from the Clinical Development of Plitidepsin) (71). Most of the patients (n=29) received the dose of 2.5 mg/day for three consecutive days, except 4 who had significant comorbidities or organ dysfunction (71). Most received only one course of therapy (n=27), except 6 patients who received a second cycle (71). Patients were elderly, with a mean (SD) age of 67.7 (9.8) years, and 75.8% had received at least one dose of COVID-19 vaccine (71). Immunocompromised status resulted from the therapy of haematologic malignancy (70% of patients), of solid tumor (27.3%), or of an antineutrophil cytoplasm antibody vasculitis (ANCA, 3%) (71). Most patients had comorbidities, including high blood pressure (30.3%), chronic lung disease (15.2%) diabetes mellitus (9.1%), and heart disease (9.1%) (71).

At onset of plitidepsin treatment, 37.9% of patients had severe pneumonia and 62.1% had moderate according to FDA classification; 48.5% were receiving oxygen supplementation; in addition to plitidepsin, 6 patients received IV immunoglobulins and 12 received sotrovimab 500 mg (anti-COVID-19 antibody) (71). The median time from initiation of plitidepsin to SpFi ≥ 315 was 8 (95% CI 7; 19) days, the median time for independence from oxygen therapy was 11 (95% CI 10; 26) days, and the median time to first negative qPCR (Ct > 36) was 17 (95% CI 13; 25) days. The mortality rate was 16% (95% CI 3; 37.3) (71).

1.3 STUDY RATIONALE

- Immunocompromised patients are at increased risk for severe and fatal coronavirus disease 2019 (COVID-19) outcomes.
- Patients with immune-system disorders have not been sufficiently represented in the clinical trials for COVID-19 antiviral drugs.
- Concerns have been raised about whether the accepted clinical practice standard to treat COVID-19 with weak antiviral agents and immunosuppressive therapies may promote chronic infection and worse outcomes in immunosuppressed patients (at risk for protracted SARS-CoV-2 infection), including selection and transmission of new SARS-CoV-2 variants.
- Studies among immunocompromised hosts should ideally be focused on identifying **direct-acting antivirals that markedly reduce SARS-CoV-2 replication**, be they small molecules or antibodies.
- Evidence of potent antiviral activity of plitidepsin observed *in vitro* and/or *in vivo*.
- Plitidepsin anti-viral activity is “agnostic” to the different SARS-CoV-2 variants.
- There is no signal supporting clinically significant immunosuppressive potential of plitidepsin neither in the context of oncology clinicalsafety analysis after the first infusion of treatment nor in the clinical COVID-19 development (APLICOV-PC study).
- The compassionate use of plitidepsin in immunosuppressed patients has shown:
  - feasibility of plitidepsin in this target population of dismal prognosis.
  - good tolerability of the 2.5 mg daily for three consecutive days.
  - a median time to SARS-CoV-2 RT-PCR negativisation of 17 days from plitidepsin Day 1.
  - a median time to oxygen independence of 13 days and, in line with this, significant radiological improvement of COVID-19 pneumonia, with exceptional requirement of high-flow oxygen intervention or intensive care unit, frequently limited in this patient population.

2. STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

- To evaluate efficacy of plitidepsin in pre-specified groups of immunocompromised patients with symptomatic COVID-19 requiring hospital care *vs* control in terms of mortality.

2.2 KEY SECONDARY OBJECTIVE

- To compare efficacy of plitidepsin *vs* the control in terms of viral clearance, in each group.

2.3 SECONDARY AND EXPLORATORY OBJECTIVES

2.3.1 Efficacy secondary objectives

- To compare efficacy of plitidepsin *vs* the control in terms of sustained end of hospital care, in each group.
- To compare efficacy of plitidepsin *vs* the control in terms of symptomatic improvement, in each group.
- To compare efficacy of plitidepsin *vs* the control in terms of clinical status (11 category WHO Clinical Progression Scale), in each group.
- To compare efficacy of plitidepsin *vs* control in terms of the need of any kind of supplementary oxygen, in each group.

2.3.2 Safety secondary objectives

- To compare safety/tolerability of plitidepsin *vs* the control in terms of adverse events, adverse reactions and mortality, in each group.
- To compare safety/tolerability of plitidepsin *vs* the control in terms of abnormal laboratory parameters, in each group.
- To compare safety/tolerability of plitidepsin *vs* the control in terms of variations of vital signs, in each group.

2.3.3 Other secondary objectives

- To compare efficacy of plitidepsin *vs* the control in the need of any type of advanced oxygen support (high-flow nasal oxygen, extracorporeal membrane oxygenation (ECMO), or non-invasive or invasive mechanical ventilation), in each group.
- To compare efficacy of plitidepsin *vs* the control in the need of intensification of respiratory or intensive care support, in each group.

- To compare efficacy of plitidepsin *vs* the control in the need of intensification of pharmacological therapies for COVID-19, in each group.
- To compare efficacy of plitidepsin *vs* control in terms of superinfection, in each group.
- To compare efficacy of plitidepsin *vs* the control in terms of all-cause- and related to COVID-19-mortality, in each group.
- To compare efficacy of plitidepsin *vs* the control in terms of subsequent hospital admissions, in each group.
- To compare efficacy of plitidepsin *vs* the control in terms of the time course of viral load, in each group.
- To compare efficacy of plitidepsin *vs* the control in terms of the evolution of inflammatory markers, in each group.
- To compare efficacy of plitidepsin *vs* the control in terms of the immune response against SARS-CoV-2, in each group.
- To compare efficacy of plitidepsin *vs* the control in terms of radiological evolution, in each group.
- To compare efficacy of plitidepsin *vs* the control in terms of restoration of the therapy for the underlying disease, in each group.
- To compare efficacy and safety/tolerability of all pooled plitidepsin arms *versus* all control arms in all the aforementioned endpoints.
- To compare efficacy and safety/tolerability between plitidepsin arms (across different groups).
- To explore prognostic/predictive factors for clinical deterioration or mortality or drug response.
- To increase pharmacology knowledge of plitidepsin.

3. OVERALL STUDY DESIGN

This is a multicentre, randomised, controlled, open-label, pragmatic, phase 2 basket study to assess the efficacy and safety of plitidepsin in different groups of immunocompromised patients with coronavirus disease 2019 (COVID-19) who require hospital care.

Approximately 150 patients are foreseen for the study, divided in 4 different groups of immunocompromised patients:

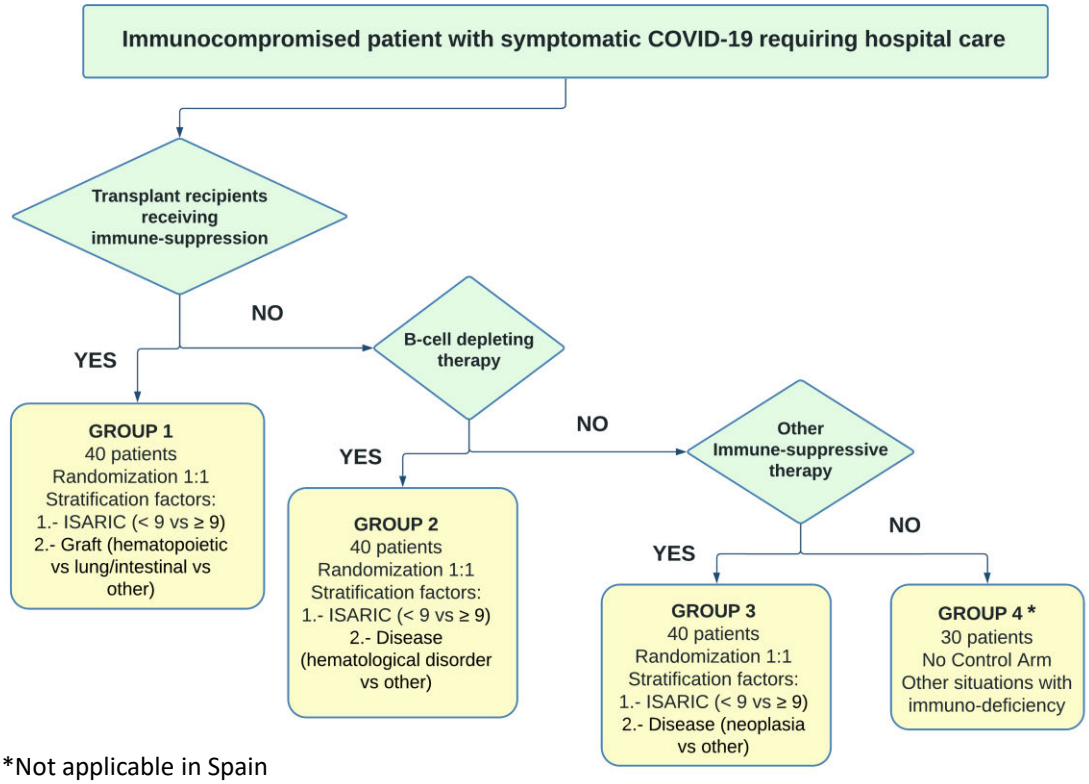
- **Group 1** –Patients receiving immune-suppression due to haematopoietic or organ transplantation**.**
- **Group 2** – Patients receiving B-cell depleting therapies.
- **Group 3** – Patients receiving other immune-suppressive therapies.
- **Group 4*** – Other situations with immune deficiencies.

*Not applicable in Spain

Whenever a patient can be allocated to more than 1 group, the priority will be group 1 > group 2 > group 3 > group 4*. In groups 1, 2, and 3, patients will be randomised to receive best standard

care (BSC) ± other antiviral (if clinically indicated) on control arm and BSC + plitidepsin on experimental arm. Group 4* will not be controlled, as shown in **Figure 9**.

*Not applicable in Spain



*Not applicable in Spain

Figure 9 Algorithm for Patient Assignment in NEREIDA Basket Trial.

Stratification:

- Group 1: ISARIC mortality (<9/≥9) | Type of graft (allogeneic haematopoietic/lung or intestinal/other)
- Group 2: ISARIC mortality (<9/≥9) | Disease (haematological neoplasm/other)
- Group 3: ISARIC mortality (<9/≥9) | Disease (neoplasia/other)
- Group 4*: Not applicable

*Not applicable in Spain

Study periods:

The study will include a Screening period, a Treatment period and a Follow-up period.

The Screening period starts once the patient has provided written informed consent and ends when the patient is randomised in the study. Parameters assessed during this time will serve as the baseline values. The screening procedures should be preferably performed within 24 h prior to administration of study treatment, allowing a maximum of 48 h prior to administration of study treatment if required.

The Treatment period starts on Day 1 (date of randomisation) to 24 h after the last dose of the antiviral therapy; if no antiviral was given in the control arm, then the treatment period will finish on Day 4, for data management purposes. Antiviral treatment must always be started on the randomisation date.

The Follow-up period starts after the end-of-treatment and lasts until the end-of-study.

The End of Study (EOS) is Day 60 (±3) or date of early study termination unless ongoing SAEs, if applicable.

The End of Trial (EOT) will be when the Last Patient Last Visit (LPLV) has occurred.

All patients will receive the best available care as per applicable local, institutional, national, supranational COVID-19 treatment guidelines.

Patients assigned to the experimental arms will receive, in addition to BSC, plitidepsin as a 60-min IV infusion, during Days 1 to 3.

Patients randomised to the control arm may receive a regulatory-approved antiviral treatment in addition to the BSC.

For prevention of infusion-related (i.e., plitidepsin-related) reactions (IRRs), allergic reactions and emesis reactions, administration of premedications is mandatory in the experimental arms.

All consenting patients discontinuing early from the study before the EOS visit should complete the early termination/withdrawal procedures.

Independent Data Monitoring Committee (IDMC)

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 not only to the study Sponsor, but also to the 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.

The 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/efficacy 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 60), to determine if there is a difference between plitidepsin and control groups and potential impact on study conduct.
- Each group will proceed with per protocol pre-established recruitment and follow-up and 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 $\geq$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 to assess the continuity of the other groups.

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

Study "Stopping rules"

The Sponsor may suspend or terminate the study if:

- New toxicological or pharmacological findings or safety issues that invalidate the earlier positive benefit-risk assessment occur;
- Significant safety concerns, according to the Sponsor's criteria, arise during the conduct of the study;
- The development of plitidepsin is discontinued;
- Any other reason (e.g., best interest of the patients, accrual rate makes the completion of the study feasible, etc.) leads to this suspension or termination.

3.1 PRIMARY ENDPOINT

- *Efficacy primary endpoint*
  - 1-month† all-cause mortality rate. †(Day 30 since randomisation).

3.2 KEY SECONDARY ENDPOINT.

- Time to confirmed negativisation in SARS-CoV-2 antigen test or RT-PCR Ct > 30.

3.3 SECONDARY ENDPOINTS

3.3.1 Efficacy secondary endpoint

- Time to sustained end of COVID-related hospital care from the time of randomisation. Sustained discharge is defined as no subsequent admission within 30 days of initial end of hospital care [up to day 60 (±3)], related to either COVID-19 or COVID-19 therapy.
- Time to sustained improvement (defined in Section 7.1.3) and resolution of selected COVID signs/symptoms (See Appendix 10 - COVID-19 signs/symptoms checklist).
- Distribution of patients according to their clinical status by the 11-category WHO Clinical Progression Scale, at Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3) (See Appendix 1 – 11-Point World Health Organization (WHO) Clinical Progression Scale).
- Percentage of patients requiring oxygen therapy on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).
- Time to sustained discontinuation (i.e., at least 7 days) of oxygen supplementation.

3.3.2 Safety secondary endpoints

Frequency of the following events (all-cause and drug-related):

- 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 related Serious Adverse Events (i.e., SARs);
- Adverse events leading to treatment discontinuation; and
- Deaths (COVID-19-related/all).
- Change respect to baseline* in individual study-defined laboratory parameters (See Section 5.7, 5.8 and 5.9 and Appendix 8 - Clinical Laboratory Analyses).
- Change respect to baseline* in individual vital signs (See Section 5.7, 5.8 and 5.9).

*Baseline = latest test performed between screening and drug administration

3.3.3 Other secondary/exploratory endpoints

- Percentage of patients requiring advanced oxygen support on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2,) and 60 (±3).
- Time to intensification of respiratory support (WHO >5) (See Appendix 1 – 11-Point World Health Organization (WHO) Clinical Progression Scale).
- Total duration of advanced oxygen support.
- Percentage of patients requiring high-flow oxygen on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).
- Total duration of high-flow oxygen therapy per patient.
- Percentage of patients requiring non-invasive mechanical ventilation on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).
- Total duration of non-invasive mechanical ventilation per patient.
- Percentage of patients requiring invasive mechanical ventilation or ECMO on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).
- Total duration of invasive mechanical ventilation or ECMO per patient
- Percentage of patients requiring admission to ICU on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).
- Total duration of intensive care unit (ICU) stay.
- Time to onset of additional (i.e., not present at baseline) immune-modulating drugs.
- Percentage of patients receiving immune-modulating drugs on Days 1, 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).
- Time to onset of additional (i.e., not present at baseline) antiviral drugs.
- Percentage of patients receiving subsequent antiviral drugs (i.e., not present at baseline) on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).
- Percentage of patients with a new infection by Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).
- Cumulative mortality (all-cause and related to COVID-19) by Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).
- Percentage of patients in each study group who require subsequent admission within 30 days of initial end of hospital care [up to Day 60(±3)], related to either COVID-19 or COVID-19 therapy.
- Change respect to Day 1 in the viral load of SARS-CoV-2, measured by RT-PCR, by Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), 45 (±2), and 60 (±3), in each study group.
- Percentage of patients in each study group with undetectable viral load, by Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), 45 (±2), and 60 (±3).
- Time to either undetectable viral load of SARS-CoV-2 or > 2 logs reduction respect to Day 1
- Change respect to baseline* in inflammatory and immunological (C- reactive protein [CRP], procalcitonin, lactate dehydrogenase [LDH], ferritin, neutrophil-to-

lymphocyte ratio, ALC, D-dimer, and multiplex cytokines assay, by Day 4 (±1), 8 (±1), 15 (±1) and 30 (±2) and 60 (±3).
- Change respect to baseline* in individual serological assessments against SARS- CoV- 2, by Days 30 (±2), and 60 (±3).
- Change respect to baseline* in individual T-cell response against SARS-CoV-2 by Days 30 (±2), and 60 (±3).
- Change respect to baseline* to Days 15 (±1), and 30 (±2) in chest X-ray findings (Brixia score, centrally assessed) (Appendix 9 – Brixia Score).
- Percentage of patients requiring modification of the therapy (drugs, dose or schedule) for the underlying disease.
- Time in which pre-scheduled therapies for the control of the underlying disease were not able to be administered.
- Risk for mortality / clinical deterioration according to individual clinical characteristics and laboratory observations present at baseline*, scores of validated scales (e.g., ISARIC-4C), as well as features related to COVID-19 [including SARS-CoV-2 variant (if available), vaccination status, duration of the infection, and inflammation-related parameters [neutrophil-to-lymphocyte ratio (NLR), protein C reactive, interleukine-6 (IL-6) and lymphocytes], the underlying disease, and other co-morbidities.
- Limited-sampling pharmacokinetics assessment (See Section 7.6).
- PK-PD analysis.

*Baseline = latest test performed between screening and drug administration

4. SELECTION OF PATIENTS

Patients must fulfill all the following inclusion/exclusion criteria to be eligible to participate in the study.

4.1 INCLUSION CRITERIA

To be eligible for inclusion into this study, each patient must fulfil the following inclusion criteria within 48 hours prior to randomisation:

General inclusion criteria:

1. Signed informed consent obtained prior to initiation of any study-specific procedures and study treatment;
2. Patient aged ≥18 years;
3. Patient diagnosed COVID-19, with the following characteristics: a) A regulatory approved test, collected no more than 3 days prior to study randomisation, with either a Ct value ≤30 or a positive antigen test;

   b) Presence of any of the selected signs/symptom listed in Appendix 10 - COVID-19 signs/symptoms checklist within the last 24 h;

4. Patient already admitted or requiring hospital care* for symptomatic COVID-19, for which at least one antiviral has failed† or cannot be used‡, after a minimum washout period of 24h for small molecules (e.g., remdesivir, molnupiravir, nirmaltrevir/ritonavir) and 5 days for antiviral monoclonal antibodies (e.g., tixagevimab + cilgavimab) or convalescent plasma.

* The definition of hospital care is based on the need to use a hospital environment (hospital ward, day hospital) for treatment administration and/or clinical monitoring of the patient with COVID-19.

† Failure of a prior antiviral is defined as a documented lack of clinical response, plus evidence of persisting positivity for SARS-CoV-2 in an appropriate biological sample, determined by a regulatory approved test, collected no more than 3 days prior to study randomisation, with either a Ct value $\leq$ 30 (RT-PCR) or a positive antigen test.

‡ Contraindication, absence of labelled indication, guidelines, or drug unavailability.

5. Adequate bone marrow, liver, kidney, and metabolic function, defined by the following tests performed at local laboratory:
    - Absolute neutrophil count ≥500/mm3 (0.5 x 10^9/L);
    - Platelet count ≥ 50 000/mm^3 (50 x 10^9/L);
    - Alanine transaminase (ALT) ≤3 x upper limit of normal (ULN) (≤5 x ULN if pre-existent liver involvement by the underlying disease);
    - Serum bilirubin ≤1.5 x ULN (or direct bilirubin <1.5 x ULN when total bilirubin is above ULN);
    - Estimated glomerular filtration rate ≥30 mL/min [CKD-EPI Creatinine Equation (2021)] (85).

6. Females of childbearing potential must have a negative serum or urine pregnancy test by local laboratory at screening and must be non-lactating.
7. Females of child-bearing potential must use highly effective contraceptive methods, while on study treatment and for 6 months after last dose of plitidepsin. Fertile males with partners of childbearing potential must use effective contraception, while on study treatment and for 6 months after last dose of plitidepsin (See Appendix 2 – Contraception and pregnancy testing). Patients in the control arm must follow contraception methods 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 highly effective (females of child-bearing potential) and effective (fertile males with partners of child-bearing

potential) contraception for at least one week after the study completion or the time indicated based on the investigator's discretion.

Group-specific inclusion criteria:

- **Group 1 – Patients receiving, within the last 30 days, immune-suppressive therapy due to haematopoietic or organ transplantation.**
  - Haematopoietic transplantation.
  - Solid Organ Transplantation:
    - Lung / intestinal.
    - Other.
- **Group 2 – Patients receiving B-cell depleting therapies within the last 6 months*.**
  Includes (but is not limited to):
  - Monoclonal antibodies (mAbs) targeting CD19, CD20, CD38, or CD52 (e.g., rituximab, ocrelizumab, ofatumumab, daratumumab, alemtuzumab).
  - B-cell activating factor (BAFF) inhibitors (e.g., belimumab).
  - Bruton's tyrosine kinase (BTK) inhibitors (e.g., evobrutinib, ibrutinib).
  - Chimeric antigen receptor T cell therapy (CAR-T) (e.g., anti-CD19 CAR-T cell).

*Time restriction is not applicable for CAR-T cell therapy.

- **Group 3 – Patients receiving, within the last 30 days, other immune-suppressive therapies.**
  - Other immunosuppressive therapies not including B-cell depleting agents for the treatment of auto-immune disorders.
  - Chemotherapy or targeted therapies with immunosuppressive potential for solid tumours or haemotological disorders.
  - Chronic glucocorticoids (i.e., equivalent to prednisone ≥ 20 mg/day for more than 1 month).
- **Group 4* – Other situations with immunodeficiency.**
  - Primary immune deficiencies.
  - Human immunodeficiency virus (HIV) infection, with $CD4^+$ T lymphocyte < 200 cells/µL in the last month.
  - Radiation therapy within the last 3 months- requires documentation of ALC < 500 cells/µL.
  - Haematological neoplasia or myelodysplasia not currently receiving any therapy
  - Other situations with a documentation of ALC < 500 cells/µL.

*Not applicable in Spain

Further information about groups and immunosuppressive therapies can be found in Appendix 12 – Definition of NEREIDA Study Groups and lists of immunosuppressive treatments.

4.2 EXCLUSION CRITERIA

1. Evidence of critical illness, defined by at least one of the following:
   - Respiratory failure defined based on resource utilization requiring at least one of the following: endotracheal intubation and mechanical ventilation, ECMO, or clinical diagnosis of severe acute respiratory distress syndrome with PaO_2*/FiO_2 ≤ 100.

     *In case a direct measure of PaO_2 has not been obtained, it should be imputed according to a referenced formula (Ellis or Rice) (Appendix 5 - Imputation of PaO_2/FiO_2 ratio). For sites located over 1000 m altitude above sea level, PaO_2/FiO2 ratio will be adjusted (Appendix 6- Adjustment of PaO_2 from a Site at High Altitude; See also Appendix 7 for FiO2 imputations from oxygen flow rates).
   - Shock requiring vasopressors.
   - Multi-organ dysfunction/failure.
2. (Criterion eliminated and merged with inclusion criterion #4 based on AEMPS recommendations).
3. Any of the following cardiac conditions or risk factors:
   - Cardiac infarction or cardiac surgery episode within the last month;
   - History of known congenital QT prolongation;
   - Known structural cardiomyopathy with abnormal LVEF (<50%);
   - Current clinical evidence of heart failure or acute cardiac ischaemia (New York Heart Association (NYHA) class III-IV).
4. Hypersensitivity to the active ingredient or any of the excipients (mannitol, macrogolglycerol hydroxystearate, and ethanol) or contraindication to receive dexamethasone, antihistamine H1/H2, or anti-serotoninergic $5HT_3$ agents.
5. Females who are pregnant or breast-feeding.
6. Females and males with partners of childbearing 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.
7. Any situation currently requiring increasing needs of immune suppressive agents (e.g. acute graft rejection, flare of autoimmune disorder, or cytokine storm syndrome).
8. 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.
9. Participation in another clinical study involving an investigational drug within 30 days prior to screening.

5. PLAN OF THE STUDY

5.1 PLANNED TRIAL PERIODS (INDIVIDUALLY PER PATIENT)

Patients will be evaluated at scheduled visits:

- The Screening period: From the day patient has provided written informed consent to randomisation. The screening procedures should be preferably performed within 24 h prior to administration of study treatment, allowing a maximum of 48 h prior to administration of study treatment if required.
- The Treatment period: From Day 1 (date of randomisation) to 24 h after the last dose of the antiviral therapy. If no antiviral was given in the control arm, then the treatment period will finish on Day 4, for data management purposes. Antiviral treatment must always be started on the randomisation date.
- The Follow-up period: From the end-of-treatment until the end-of-study.
- The End of Study (EOS) is Day 60 (±3) or date of early study termination unless ongoing SAEs, if applicable.

5.2 DISCONTINUATIONS

5.2.1 Screening Failure

Screen failures are defined as patients who consent to participate in the clinical study but are not subsequently randomised 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.

5.2.2 Treatment Discontinuation

Treatment discontinuation occurs when an enrolled patient ceases to receive the study treatment regardless of the circumstances.

The primary reason for any treatment discontinuation will be recorded on the patient's electronic Case Report Form (e-CRF).

A subject may stop the study treatment but desirably should continue to the end of study. Should a patient decide to prematurely discontinue the study treatment (refuses treatment), all efforts will be made to complete and report the observations as thoroughly as possible. He/she should be asked if he/she can still be contacted for further information. The outcome of that discussion should be documented in the medical records. Whenever possible, 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.

Patients will receive the study treatment(s) while it is considered to be in their best interest. Specifically, individual treatment of a given patient will be prematurely ended if:

- Unacceptable toxicity despite allowed/applicable medical prophylaxis or therapy.
- Intercurrent illness of sufficient magnitude to preclude safe continuation of the study treatment.
- Patient's refusal (follow-up has to be continued for those patients who refuse treatment, but do not withdraw consent) and/or non-compliance with study requirements.
- A major protocol deviation that may affect the risk/benefit ratio for the participating patient.
- Investigator's decision.

A rapid disease progression per se is not an indication for treatment discontinuation and needs additional clinical input. Patients who are withdrawn for any reason must not be re-treated in the context of this study at any time.

All cases of treatment discontinuation must be documented (date and reason) in the eCRF. Patients will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and are not obliged to state their reasons.

5.2.3 Study Discontinuation

Study discontinuation occurs when an enrolled patient ceases to participate in the study, regardless of the reason. Patients will remain on study until patient consent withdrawal, death, or the date of study termination (clinical cutoff) established by the Sponsor. The date and reason for study discontinuation will be clearly documented in the medical records of the patient.

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 13) for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

Reasons for study discontinuation can include, but are not limited to:

- Investigator's decision;
- Patient refusal as reason for discontinuation.

5.2.4 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), 15 (±1), 30 (±2), and 60 (±3), 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:

- 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 lost to follow-up.

5.3 PROTOCOL DEVIATIONS

A protocol deviation is defined as any departure from what is described in the protocol of a clinical trial approved by an Independent Ethics Committee (IEC)/Institutional Review Board (IRB) and Competent Authorities. Therefore, it applies to deviations related to patient inclusion and clinical procedures (e.g., assessments to be conducted or parameters to be determined), and also to other procedures described in the protocol that concern the Good Clinical Practice (GCP) guidelines or ethical issues (e.g., issues related to obtaining the patients' Informed Consent, data reporting, the responsibilities of the Investigator, etc.).

Deviations with no effects on the risk/benefit ratio of the clinical trial (such as minimal delays in assessments or visits) will be distinguished from those that might have an effect on this risk/benefit ratio, such as:

- Deviations that might affect the clinical trial objectives, such as those involving the inclusion/exclusion criteria (which could mean that the patient is not eligible for the trial) and those having an effect on patient evaluability.
- Deviations that might affect the patient's well-being and/or safety, such as an incorrect dosing of the study treatment due to not following dose adjustment specifications or an incorrect preparation of the medication.
- Deviations related to the following of GCP guidelines as described in the protocol and regulations in force, such as deviations when obtaining the Informed Consent or not following the terms established for reporting SAEs, etc.

No deviations that may have an effect on the risk/benefit ratio of the clinical trial will be authorized. All protocol deviations detected during the study will be appropriately documented, and those considered particularly relevant (i.e., those related to ethical issues, to fulfillment of GCP guidelines and with an effect on the risk/benefit ratio) will be notified, if applicable, to the pertinent IEC/IRB and to the Competent Authorities as established by local regulations.

5.4 REPLACEMENT OF PATIENTS

No patients will be replaced. For each group 1-3, all efficacy analyses will be done on Full Analysis Set (FAS) population (See 8.2.1 Full Analysis Set (FAS) population).

Therefore, accrual in each group will end when the target number of evaluable patients in the FAS population has been reached.

5.5 PATIENT SCREENING

The Screening period starts once the patient has provided written informed consent and ends when the patient is randomised/enrolled in the study.

5.5.1 Screening Assessments and procedures

Parameters assessed during this time may serve as the baseline values (unless repeated before Day 1 treatment). The patient will be screened with the aim to complete screening within 24 h prior to administration of study treatment but allowing a maximum of 48 h prior to administration of study treatment if required. Upon confirmation of eligibility, the patient will be randomised and start treatment on the same day.

	PROCEDURES / ASSESSMENTS
Patient Registration	• ICF signature.
Demographics	• Demographic data: gender, year of birth, age and race, according to the local regulation.
Medical and immunodeficiency history.	• Medical History • Underlying disease causing immune deficiency. • Concomitant therapies. • SARS-CoV-2 Vaccination status (brand and dates of vaccines) • Prior medications for COVID-19. • Scheduled therapies for the underlying disease and compliance. A detailed history of medications and procedures will be documented for each patient at screening. Any of the medications ongoing before the first dose of the study treatment will be recorded, including start/stop dates, indication, dose and frequency. Specific focus will be done on medications used to treat COVID-19 or the underlying disease, including the theoretical schedule for next coming administrations

	PROCEDURES / ASSESSMENTS
Clinical evaluation	• COVID-19-related signs and symptoms checklist (Appendix 10 - COVID-19 signs/symptoms checklist) and need for hospitalisation. • Complete physical examination. Height in centimetres (cm) will be measured at screening. Body weight will be measured in kilograms (kg). Body mass index will be calculated. A full physical examination will be performed and should include general appearance, skin, neck, eyes, throat, lungs, heart, abdomen, back, lymph nodes, extremities, and neurological examinations, including Glasgow score (See Appendix 11 – Glasgow Coma Scale). • Clinical status (11-point WHO Clinical Progression Scale) • Vital signs will include body temperature (°C), SBP (mmHg), DBP (mmHg), pulse rate (bpm) and respiratory rate (breaths/min). • Lung function tests include partial pressure of oxygen (PaO_2), fraction of inspired oxygen (FiO_2) and oxygen saturation (SpO_2). PaO_2/FiO_2 ratio will also be measured; either Ellis or Rice formulae can be used to estimate PaO_2/FiO_2 ratio (Appendix 5 - Imputation of PaO_2/FiO_2 ratio).
Detection of SARS-CoV-2	• qRT-PCR SARS-CoV-2 or SARS-CoV-2 Rapid Antigen Test (RAT) Documented diagnosis of SARS-CoV-2 infection, determined by a regulatory approved test, collected no more than 3 days prior to study randomisation, with either a Ct value ≤30 or a positive antigen test. SARS-CoV-2 variant will be documented at any time during the study, if available information exists.
Minimum set of laboratory tests for screening (at site's laboratory)	**Haematology:** differential white blood cells (WBC) counts (including neutrophil and lymphocyte counts), platelet count (See Appendix 8 – Clinical Laboratory Analyses). **Biochemistry:** Liver function test (ALT, total bilirubin (direct bilirubin if total is abnormally elevated), calculated CrCL [as per Chronic Kidney Disease Epidemiology Collaboration (CKD-

	PROCEDURES / ASSESSMENTS
	EPI)], C-reactive protein (CRP), blood urea nitrogen (BUN)/urea (See Appendix 8 – Clinical Laboratory Analyses).
ECG	A 12-lead ECG will be performed in triplicate. Cardiac rhythm will be identified in ECG intervals of at least 30 seconds of duration. PR interval, QT interval (raw and QTcF), heart rate and QRS complex, with specialist assessment/judgment of further evaluation if appropriate.
Pregnancy test (if patient is a WOCBP)	A urine or serum pregnancy test will be performed for female patients of childbearing potential (i.e., female patients who are not postmenopausal or surgically sterile) at screening.
Eligibility assessment	Documented review of all Inclusion and Exclusion criteria.
ISARIC-4C mortality score	Appendix 4
Adverse events	AEs that occurred after signature of the informed consent form will be reported.

The patient will be a screening failure if not all inclusion criteria are met or if any exclusion criterion is met. A patient should only be treated if eligibility criteria are still met according to assessments performed closest to first drug administration.

5.6 PATIENT RANDOMISATION

Patients will be sequentially allocated in their respective group, following the algorithm depicted in **Figure 9**.

Central randomisation will be implemented. In groups 1-3, patients will be assigned to the experimental or the control arms at a 1:1 ratio. Stratification will be performed in the randomised groups. The ISARIC-4C mortality score will be used as a stratification factor in all groups (score <9/≥9). In addition, the type of graft (allogeneic haematopoietic/lung or intestinal/other) will be used for Group 1, whereas the underlying disease will be considered for Groups 2 and 3. For Group 2, the factor categories will be haematological neoplasm *vs* other disease; the respective categories for Group 3 will be neoplasia *vs* other. Stratification is not applicable in the non-randomised Group 4.

Day 1 is defined as the day of randomisation. Treatment must start within the same natural day.

5.7 EVALUATIONS AND PROCEDURES DURING TREATMENT

	ASSESSMENT/PROCEDURES	TIME
Clinical evaluation	• COVID-19-related signs and symptoms checklist (Appendix 10 – COVID-19 signs/symptoms checklist). • Clinical status (11-point WHO Clinical Progression Scale). • Vital signs will include body temperature (°C), SBP (mmHg), DBP (mmHg), pulse rate (bpm) and respiratory rate (breaths/min). • Targeted physical examination. A targeted physical examination will include heart, lungs, abdomen, extremities plus central nervous system (CNS) assessment, based on the investigator's decision as per clinical evolution. • Lung function tests include PaO_2, FiO_2 and SpO_2. PaO_2/FiO_2 ratio will also be measured; either Ellis or Rice formulae can be used to estimate PaO_2/FiO_2 ratio (Appendix 5 - Imputation of PaO_2/FiO_2 ratio).	Days 1, 2, 3, 4 (±1). When applicable, these assessments should be performed before treatment administration.

	ASSESSMENT/PROCEDURES	TIME
	• Concomitant therapies. • Subsequent medications for SARS-CoV-2 infection. • Subsequent medications for the underlying disease.	Days 1, 2, 3, 4.
Laboratory tests*	**Haematology:** haematocrit, red blood cells (RBC), differential WBC counts (including neutrophil and lymphocyte counts), platelet count and haemoglobin (See Appendix 8 – Clinical Laboratory Analyses).	Days 1, 4 (±1), and whenever clinically indicated for an AE evaluation. When applicable, these assessments should be performed before treatment administration.
	Biochemistry: Liver function test [ALT, AP, AST[J], albumin, total bilirubin (direct bilirubin if total is abnormally elevated)], lactate dehydrogenase (LDH), glucose [fasting], sodium, potassium, calcium, BUN/urea, gamma glutamyl transferase (GGT), creatinine, calculated creatine clearance [CKD-EPI], creatine phosphokinase (CPK), amylase, lipase, pro-calcitonin, ferritin, and CRP (See Appendix 8 – Clinical Laboratory Analyses).	Days 1, 4 (±1), and whenever clinically indicated for an AE evaluation. When applicable, these assessments should be performed before treatment administration.
	Coagulation: D-Dimer (See Appendix 8 – Clinical Laboratory Analyses).	Day 1, 4 (±1), and whenever clinically indicated for AE evaluation. When applicable, these assessments should be performed before treatment administration.
	Urine analysis (semi-quantitative elemental tests	Day 1 (previous to treatment administration). Repeat whenever

	ASSESSMENT/PROCEDURES	TIME
	for specific gravity, blood, pH, proteins, bilirubin, glucose, ketones, nitrites, and qualitative analysis of the sediment).	clinically indicated for AE evaluation. When applicable, these assessments should be performed before treatment administration.
Inflammatory and immunological biomarkers in blood (central laboratory)	Luminex® Multiplex cytokine assessment.	Days 1, 2, 4 (±1).
Anti-SARS-CoV-2 (central laboratory)	IgG/M anti-protein S and N.	Day 1 previous to treatment administration.
	T-cell response (IFN-γ) to SARS-CoV-2 S-protein (Quantiferon®).	
Detection of SARS-CoV-2	qRT-PCR in oro/naso-pharyngeal exudates (central laboratory).	Day 1, 4 (±1).
	Rapid Antigen Test (at site).	Day 1, 4 (±1).
ECG**	Cardiac rhythm will be identified in ECG intervals of at least 30 seconds of duration, PR interval, QT interval (raw), heart rate and QRS complex, with specialist assessment/judgment of further evaluation if appropriate.	At screening or Day 1 (previous to treatment administration). Repeat on Day 4 (±1), and whenever clinically indicated for AE evaluation.
Chest X-ray	Qualitative evaluation and Brixia score (See Appendix 9 – Brixia Score). (Submission to central review)	At screening or Day 1 (previous to treatment administration). Repeat whenever clinically indicated for AE evaluation.
ISARIC-4C deterioration score	Appendix 4	At screening or Day 1 (previous to treatment administration).
Pharmacokinetics (central laboratory)	(See details in Section 7.6).	Days 1, 3, 4 (see details in Section 7.6).
Antiviral Treatment administration	(See details in Section 6.1).	Days 1-3 in the experimental arm.¥

	ASSESSMENT/PROCEDURES	TIME
		From Day 1 (date of randomisation) to the last dose of the antiviral therapy in the control arm.[¥]
Survival status		Throughout the treatment period.
Hospitalisation status		Throughout the treatment period.
Adverse events	As per NCI-CTCAE v.5***	Throughout the treatment period.

* Central laboratory for all the tests, except for screening.

** Triplicate.

*** The "AE form" will be used to report signs and symptoms and medical history in case of any significant change (improvement or worsening) during the study, as well as for events that occur after the first drug infusion or any event related to a study procedure within the study period (according to ICH guidelines).

[¥]Antiviral treatment period in both arms will end 24 h after administration of the last dose of the antiviral therapy. If no antiviral was given in the control arm, then the treatment period will finish on Day 4.

[ʃ]See Section 7.2.1 Adverse Events of Special Interest (AESIs).

For patients included in the study (all randomised patients) but never treated, only the applicable forms at baseline and off-study visit modules of the e-CRF should be completed but SAEs occurring during screening period need to be reported (using paper SAE forms).

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 follow-up to the end of study.

5.8 EVALUATIONS DURING FOLLOW-UP

	ASSESSMENT/PROCEDURES	TIME
Clinical evaluation	• COVID-19-related signs and symptoms checklist (Appendix 10 – COVID-19 signs/symptoms checklist).	Daily while admitted, upon discharge, Day 8 ± 1 Day 15 ± 1 Day 30 ± 2 Day 45[¥] ± 2 and if readmission.

	ASSESSMENT/PROCEDURES	**TIME**
	• Clinical status (11-point WHO Clinical Progression Scale) • Vital signs will include body temperature (°C), SBP (mmHg), DBP (mmHg), pulse rate (bpm) and respiratory rate (breaths/min). • Targeted physical examination. A targeted physical examination will include heart, lungs, abdomen, extremities plus CNS assessment, based on the investigator's decision as per clinical evolution. • Lung function tests include PaO_2, FiO_2 and SpO_2. PaO_2/FiO_2 ratio will also be measured; either Ellis or Rice formulae can be used to estimate PaO_2/FiO_2 ratio (Appendix 5 - Imputation of PaO_2/FiO_2 ratio).	¥Only assessment of COVID-19-related signs and symptoms checklist
	• Concomitant therapies. • Subsequent medications for SARS CoV 2 infection. • Subsequent medications for the underlying disease.	Throughout follow-up period, if changes
Laboratory tests*	**Haematology:** haematocrit, RBC, differential WBC counts	Day 8 ± 1

	ASSESSMENT/PROCEDURES	TIME
	(including neutrophil and lymphocyte counts), platelet count and haemoglobin (See Appendix 8 – Clinical Laboratory Analyses).	Day 15 ± 1 Day 30 ± 2 If clinically indicated (AE), and if readmission
	Biochemistry: Liver function test [ALT, AP, AST[J], albumin, total bilirubin (direct bilirubin if total is abnormally elevated)], LDH, glucose [fasting], sodium, potassium, calcium, BUN/urea, GGT, creatinine, calculated creatine clearance [CKD-EPI], CPK, amylase, lipase, pro-calcitonin, ferritin, and CRP (See Appendix 8 – Clinical Laboratory Analyses).	
	Coagulation: D-Dimer (See Appendix 8 – Clinical Laboratory Analyses).	
	Urine analysis (semi-quantitative elemental tests for specific gravity, blood, pH, proteins, bilirubin, glucose, ketones, nitrites, and qualitative analysis of the sediment).	Repeat whenever clinically indicated for AE evaluation.
Inflammatory and immunological biomarkers in blood (central laboratory)	Luminex® Multiplex cytokine assessment.	Day 8 ± 1 Day 15 ± 1 Day 30 ± 2
Anti-SARS-CoV-2 (central laboratory)	IgG/M anti-protein S and N.	Day 30 ± 2
	T-cell response (IFN-γ) to SARS-CoV-2 S-protein (Quantiferon®).	

	ASSESSMENT/PROCEDURES	TIME
Detection of SARS-CoV-2	qRT-PCR in oro/naso-pharyngeal exudates (central laboratory).	Day 8 ± 1 Day 15 ± 1 Day 30 ± 2 Day 45 ± 2
	Rapid Antigen Test (at site).	Every other day while admitted, Day 8 ± 1 Day 15 ± 1 Day 30 ± 2 Day 45 ± 2
ECG**	Cardiac rhythm will be identified in ECG intervals of at least 30 seconds of duration, PR interval, QT interval (raw), heart rate and QRS complex, with specialist assessment/judgment of further evaluation if appropriate.	Whenever clinically indicated for AE evaluation.
Chest X-ray	Qualitative evaluation and Brixia score (See Appendix 9 – Brixia Score). (Submission to central review)	Day 15 ± 1 Day 30 ± 2
Pharmacokinetics (central laboratory)	(See details in Section 7.6).	Day 8 (±1) (see details in Section 7.6).
Survival status		Throughout the treatment period
Hospitalisation status		Throughout the treatment period
Adverse events	As per NCI-CTCAE v.5***	Throughout the treatment period.

* Central laboratory for all the tests

** Triplicate.

*** The "AE form" will be used to report signs and symptoms and medical history in case of any significant change (improvement or worsening) during the study, as well as for events that occur after the first drug infusion or any event related to a study procedure within the study period (according to ICH guidelines).

[ʃ]See Section 7.2.1 Adverse Events of Special Interest (AESIs).

5.9 EVALUATIONS AT THE END OF STUDY

The following assessments will be performed at Day 60 ± 3 or at the date of early study termination:

	ASSESSMENT/PROCEDURES
Clinical evaluation	• COVID-19-related signs and symptoms checklist (Appendix 10 – COVID-19 signs/symptoms checklist). • Clinical status (11-point WHO Clinical Progression Scale). • Vital signs will include body temperature (°C), SBP (mmHg), DBP (mmHg), pulse rate (bpm) and respiratory rate (breaths/min). • Targeted physical examination. A targeted physical examination will include heart, lungs, abdomen, extremities plus CNS assessment, based on the investigator's decision as per clinical evolution. Body weight will be measured in kilograms (kg). • Lung function tests include PaO_2, FiO_2 and SpO_2. PaO_2/FiO_2 ratio will also be measured; either Ellis or Rice formulae can be used to estimate PaO_2/FiO_2 ratio (Appendix 5 - Imputation of PaO_2/FiO_2 ratio).
	• Concomitant therapies. • Subsequent medications for SARS-CoV-2 infection. • Subsequent medications for the underlying disease.
Laboratory tests*	**Haematology:** haematocrit, RBC, differential WBC counts (including neutrophil and lymphocyte counts), platelet count and haemoglobin (See Appendix 8 – Clinical Laboratory Analyses).
	Biochemistry: Liver function test [ALT, AP, AST[ʃ], albumin, total bilirubin (direct bilirubin if total is

	ASSESSMENT/PROCEDURES
	abnormally elevated)], LDH, glucose [fasting], sodium, potassium, calcium, BUN/urea, GGT, creatinine, calculated creatine clearance [CKD-EPI], CPK, amylase, lipase, pro-calcitonin, ferritin, and CRP (See Appendix 8 – Clinical Laboratory Analyses).
	Coagulation: D-Dimer (See Appendix 8 – Clinical Laboratory Analyses).
	Urine analysis (semi-quantitative elemental tests for specific gravity, blood, pH, proteins, bilirubin, glucose, ketones, nitrites, and qualitative analysis of the sediment).
Inflammatory and immunological biomarkers in blood (central laboratory)	Luminex® Multiplex cytokine assessment.
Anti-SARS-CoV-2 (central laboratory)	IgG/M anti-protein S and N.
	T-cell response (IFN-γ) to SARS-CoV-2 S-protein (Quantiferon®).
Detection of SARS-CoV-2	qRT-PCR in oro/naso-pharyngeal exudates (central laboratory).
	Rapid Antigen Test (at site, provided by the Sponsor).
ECG**	Only if clinically indicated. Cardiac rhythm will be identified in ECG intervals of at least 30 seconds of duration, PR interval, QT interval (raw), heart rate and QRS complex, with specialist assessment/judgment of further evaluation if appropriate.
Chest X-ray	Qualitative evaluation and Brixia score (Appendix 9 – Brixia Score). (Submission to central review)
Survival status	
Hospitalization status	
Adverse events	As per NCI-CTCAE v.5***

* Central laboratory for all the test

** Triplicate.

*** The "AE form" will be used to report signs and symptoms and medical history in case of any significant change (improvement or worsening) during the study, as well as for events that occur after the first drug infusion or any event related to a study procedure within the study period (according to ICH guidelines).

[See Section 7.2.1 Adverse Events of Special Interest (AESIs).

6. TREATMENTS

6.1 DESCRIPTION OF TREATMENTS

6.1.1 Drug Formulation and Supply

Plitidepsin 2 mg powder for concentrate for solution for infusion is presented as a sterile, preservative free, white to off-white lyophilised powder/cake in a 10 mL, single-dose, Ph. Eur. type I clear-glass vial.

[redacted]

Before use, the powder/cake is reconstituted with 4 mL of solvent for plitidepsin to give a solution containing plitidepsin 0.5 mg/mL. The reconstituted solution is diluted further in either sodium chloride 9 mg/mL (0.9%) solution for infusion (normal saline solution, NSS) or glucose 50 mg/mL (55) solution for infusion (D5W) for IV infusion.

6.1.2 Storage conditions

Plitidepsin powder, 2 mg, must be stored at 5°C ± 3°C (2°C to 8°C). Keep the vials in the outer carton to protect from light.

Solvent for plitidepsin must be stored at 5°C ± 3°C (2°C to 8°C).

6.1.3 Administration of Study Medications

The IMP may be administered only by the Investigator or by a member of staff specifically authorised by the Investigator, as appropriate.

6.1.4 Treatment Schedule and Dose

Plitidepsin will be administered as a 60-min (±5 min) IV infusion, every 24 h (±30 min)* for 3 consecutive days, at a dose of 2.5 mg.

For prevention of infusion-related (i.e., plitidepsin-related) reactions (IRRs), allergic reactions and emesis reactions, administration of premedications is mandatory in the experimental arms.

*Treatment windows do not apply for the first 30 patients enrolled in this study (experimental arm) who will be included in the pharmacokinetics evaluations.

6.1.5 Prophylactic Medication

For prevention of plitidepsin related infusion reactions, the following premedications MUST be given to each patient and should be completed no more than ~2 hours to 20 minutes before initiating each plitidepsin infusion:

- Dexamethasone phosphate* 8 mg IV (equivalent to 6.6 mg of dexamethasone base);
- Dexchlorpheniramine maleate* 5 mg IV (or diphenhydramine hydrochloride 25 mg IV);
- Famotidine* 20 mg IV (or equivalent such as ranitidine 50 mg IV). Oral famotidine is acceptable, but in this case the dose should be 40 mg and it must be administered 2 hours (±15 min) before plitidepsin;
- Palonosetron* 0.25 mg IV (tropisetron 5 mg IV could be considered if palonosetron is not available. It should be administered orally (PO)/IV on Days 4 and 5 if tropisetron 5 mg was administered on Days 1, 2 and 3).

*Provided by the Sponsor, who is also in charge of the labeling (except when the site uses its own medication for the trial).

6.2 DOSE REDUCTION

Not applicable

6.3 CONCOMITANT MEDICATION

6.3.1 Allowed Medications/Therapies

Any medications, with the exceptions noted in Section 6.3.2 below, which are considered necessary for the patient's welfare, and which will not interfere with the study medication, may be given at the discretion of the Investigator. Medications which MUST be administered as part of the study treatment schedule are listed in Section 6.1.5.

Administration of **all concomitant drugs** received by the patient during the "on-treatment" period of the trial must be reported in the appropriate section of the eCRF along with dosage information, dates of administration and reasons for use. Additionally, any unplanned diagnostic, therapeutic or surgical procedure performed during the study period must be recorded in the eCRF.

6.3.2 Prohibited Medications/Therapies

- Other investigational agents.
- Concomitant use of plitidepsin and antiviral treatments for COVID-19.

- Strong CYP3A4 inhibitors and inducers throughout plitidepsin treatment period and until 24-h washout period (Appendix 3 - Inhibitors and Inducers of CYP3A4), unless medical judgement that they are for the patient's best interest.

Investigator may decide to stop the study treatment at any time after the first dose of plitidepsin and initiate other antiviral treatments for COVID-19.

6.3.3 Drug-drug Interactions with Plitidepsin

There have been no clinical studies on drug interactions. Effect of other medicinal products on plitidepsin include:

Interactions with CYP3A4 inhibitors and inducers: *In vitro* studies indicate that CYP3A4 is the major enzyme involved in plitidepsin metabolism, followed by CYP2A6 and CYP2E1. However, a pharmacokinetic population analysis of plitidepsin including 283 patients indicated that concomitant administration of CYP3A4 inhibitors and inducers does not affect exposure to plitidepsin. Nevertheless, it cannot be ruled out that concomitant treatments that inhibit or induce CYP3A4 may modify the efficacy and/or increase the probability of side effects associated with plitidepsin, so they should be avoided.

- Co-administration of plitidepsin with strong CYP3A4 inhibitors and inducers should be avoided, as they may affect the plasma concentration of plitidepsin, unless medical judgement that they are for patient's best interest.
- Co-administration with moderate CYP3A4 inhibitors and inducers should be used with caution, as an effect on plitidepsin exposure cannot be excluded.

Interactions with CYP3A4 inhibitors: Plitidepsin should not be administered with potent inhibitors CYP3A4 (e.g. grapefruit juice, clarithromycin, itraconazole, nefazodone, telithromycin, voriconazole). Potent inhibitors CYP3A4 should be discontinued before starting treatment and during treatment with plitidepsin. They may be re-administered 24 hours after the last dose of plitidepsin.

Moderate inhibitors CYP3A4 (e.g., aprepitant, diltiazem, erythromycin, fluconazole, verapamil) should be used with caution, as increased exposure to plitidepsin cannot be excluded

Interactions with CYP3A4 inducers: To avoid a decrease in the effectiveness of plitidepsin, plitidepsin should not be administrated with potent inducers of CYP3A4 enzyme, such as anticonvulsants (phenytoin, phenobarbital or carbamazepine), rifampicin, rifabutin and St.John's wort, unless there are no therapeutic alternatives. The appropriate initial dose for patients taking potent inducers has not been defined.

They may be administered again 24 hours after the last dose of plitidepsin. CYP3A4 moderate inducers (e.g., bosetan, modafinil, nafcillin) should be used with caution, as a reduction of exposure to plitidepsin cannot be excluded. A list of CYP3A4 inducers and inhibitors is included in Appendix 3 - Inhibitors and Inducers of CYP3A4.

Effect of plitidepsin on other drugs: In vitro studies did not show a potential for plitidepsin to inhibit or induce metabolism of other drugs. However, the results for ruling out the potential inducing effect of plitidepsin on the CYP2B6 enzyme were inconclusive. The potential effect of

plitidepsin on CYP2B6 has not been studied *in vivo*. Therefore, decreases in plasma concentrations of drugs that are substrates of this enzyme cannot be ruled out when co-administered with plitidepsin.

The use of any herbal/natural products or other "folk remedies" should be discouraged, but use of those products, as well as the use of vitamins, nutritional supplements, and all other concomitant medications must be recorded in the eCRF.

6.4 DRUG ACCOUNTABILITY

Proper drug accountability will be done by the appropriate trained study personnel. Each study site will keep records to allow a comparison of quantities of drug received and used at each site for monitoring purposes. The Investigator at each study site will be the person ultimately responsible for drug accountability at the site.

All unused drug supplied by the Sponsor will be properly destroyed at the study site. Documentation of this procedure must be provided to the clinical trial monitor. If the Sponsor agrees, unused drug supplies may be returned to the drug repository.

6.5 TREATMENT COMPLIANCE

The Investigator is ultimately responsible for supervising compliance with the instructions described in this study protocol.

7. STUDY EVALUATIONS

7.1 EFFICACY

7.1.1 Test for COVID-19

A SARS-CoV-2 Rapid Antigen Test, provided by the Sponsor, will also be performed at the site by the research team on Day 4 until discharge, and always at Days 1, 4 (±1 day), 8 (±1 day), 15 (±1 day), 30 (±2 days), 45 (±2 days), and 60 (±3 days); if RAT is positive at discharge, RAT should also be monitored on a weekly basis until negative. A negative result should be confirmed with a second RAT within 48 h, by the investigator team.

Quantitative PCR to assess viral load on Day 1 before initiation of treatment and on Days 4 (±1 day), 8 (±1 day), 15 (±1 day), 30 (±2 days), 45 (±2 days), and 60 (±3 days) (see Appendix 13 - Schedule of Assessments and Procedures), will be performed by a central laboratory. Based on these assessments, changes in viral load respect to Day 1 will be calculated for each patient. The proportion of patients with either > 2 log 10 reductions or undetectable SARS-CoV-2 on each time point 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 X-ray on Day 1, 15 (±1 day), and 30 (±2 days) and submitted to central review. 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 and scored using Brixia criteria (86).

7.1.3 Clinical Status

Clinical status (including the need for oxygen supplementation) will be assessed at least once a day, or whenever it changes, while receiving hospital care and on Days 4 (±1 day), 8 (±1 day), 15 (±1 day), 30 (±2 days), 45 (±2 days) and 60 (±3 days), using the 11-point WHO Clinical Progression Scale (Appendix 1 – 11-Point World Health Organization (WHO) Clinical Progression Scale).

COVID-19 signs or symptoms will be systematically collected and scored according to a check list (Appendix 10 - COVID-19 signs/symptoms checklist) together with the WHO Clinical Status. Research team will record a daily severity rating of their symptom severity over the past 24 hours based on NCI-CTCAE v5.0 criteria. Sense of smell and sense of taste will each be rated on a 3-point Likert scale where: “0” is reported if the sense of smell/taste was the same as usual, “1” if the sense of smell/taste was less than usual, and “2” for no sense of smell/taste.

Sustained improvement of all targeted COVID-19 signs/symptoms is defined as the event occurring on the first of 4 consecutive days when all symptoms scored as NCI-CTCAE category of moderate-severe intensity, or requiring medical intervention, or limiting instrumental ADL (see Appendix 10 – COVID-19 signs/symptoms checklist) are scored as mild or absent AND all symptoms scored mild or 0 (absent) at study entry are scored as 0.

The first day of the 4 consecutive-day period will be considered the First Event Date.

Sustained resolution is defined as when all targeted symptoms are scored as 0 for 4 consecutive days. The first day of the 4 consecutive-day period will be considered the First Event Date.

For any symptoms not present at baseline, it must be absent at Day 30 (+/-2) to be counted as sustained alleviation/resolution.

7.1.4 Other Efficacy Assessments

The study day (from Day 1 to Day 60 (±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
- Discontinuation of antivirals or immune-modulating drugs
- ICU admission
- ICU discharge
- Subsequent hospital 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 30 (±2 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
- Occurrence of new 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 new 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, cytokine multiplex Luminex® panel, including IL-1β, IL-6, IL-10, and TNFα) in each study arm from baseline (Day 1 prior to start of the study treatment) to on Days 4 (±1 day), 8 (±1 day), 15 (±1 day), 30 (±2 days), and 60 (±3 days),
- Anti-SARS-CoV-2 serologic and cellular response will be centrally assessed by validated tests, on Days 1, 30 (±2 days), and and 60 (±3).

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 7 - 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.

Patients will be evaluable for safety if they have received any partial or complete treatment administration.

All adverse events (AE) and adverse reactions (ARs), based on clinical signs and symptoms and laboratory measurements, will be measured daily until Day 60 (±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 7.4.2).

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.

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.

All AEs will be graded according to the NCI-CTCAE v.5. Treatment modifications and reason for treatment discontinuation will be monitored throughout the study. The latest MedDRA dictionary version will be used for AE coding.

The safety profile of patients will be monitored throughout the treatment and at Day 60 (±3 days), or until the date of death, whichever occurs first.

Any treatment-related AEs, treatment-emergent adverse events of special interest, and SAES 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.

7.2.1 Adverse Event of Special Interest

The following AEs will be considered and monitored as AEs of special interest (AESIs): musculoskeletal disorders, CPK increases, and rhabdomyolysis; hypersensitivity reactions, cardiac events and transaminase elevations, thromboembolic events, hepatobiliary disorders, and healthcare-associated infections (87).

If at any time AST/ALT levels are greater than 3 x ULN (> 5 x ULN if pre-existent liver involvement at baseline by the underlying disease, with baseline ALT levels between 3-5 x ULN):

- Report as an AESI.
- The investigator should ensure that serum liver enzyme tests, serum bilirubin, and coagulation tests (INR or equivalent) are repeated two or three times weekly (local labs). Frequency of re-testing can decrease to once a week or less if abnormalities stabilise or the trial drug has been discontinued and the subject is asymptomatic.
- The list of concomitant medications should be re-checked, and include nonprescription drugs, herbal and supplementary preparations, alcohol and recreational substances.
- Other reasons for liver injury should be ruled out, including co-infection with other virus, autoimmune or alcoholic hepatitis, non-alcoholic liver steato-hepatitis, biliary tract disease, progression of an underlying disease (e.g. liver metastasis), hypoxic/ischemic hepathopathy, or exposure to environmental agents.
- The investigator should consider additional tests to evaluate liver function or structure, as well as a consultation to specialists in liver diseases.

Treatment with plitidepsin must be discontinued in case of:

- ALT or AST > 8 x ULN;
- ALT or AST > 3 x ULN and either total bilirubin >2 x ULN or INR >1.5;
- ALT or AST > 3 x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%).

7.3 ADVERSE EVENTS DEFINITIONS

7.3.1 Adverse Event (AE)

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 unfavorable 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 [ECG]) 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 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 SAE (see definition below), and/or
- The test result is considered to be clinically relevant by the Investigator.

For the purpose of this protocol, disease progression or worsening of the underlying disease should not be reported as AEs. This information will be used only for efficacy assessment.

7.3.2 Serious Adverse Event (SAE)

A SAE is 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,
- Is medically significant, or
- Is any suspected transmission of an infectious agent via a medicinal product.

Medical and scientific judgment 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.

7.3.3 Death

Death as such is the outcome of a 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.

7.3.4 Life-threatening Event

A life-threatening event is defined as any event in which the patient 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.

7.3.5 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 a SAE unless exempted from SAE reporting (see Section 7.4.2). 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:

a. Reasons described in protocol (e.g., IMP) administration, protocol-required intervention/investigations]. However, events requiring hospitalisations or prolongation of hospitalisation as a result of a complication of therapy administration or clinical trial procedures will be reported as SAEs.
b. Hospitalisation or prolonged hospitalisation for technical, practical or social reasons, in absence of an AE.
c. 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 a SAE.

Other situation that MUST NOT be considered as hospitalisations is the following:

d. Planned treatments at sites not associated to a hospital and generally considered as minor surgical procedures (i.e., laser eye surgery, arthroscopy, etc.).

7.3.6 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. The Sponsor will use the reference safety information table included in the most updated IB for plitidepsin for the evaluation of listedness/expectedness of adverse events.

7.3.7 Adverse Reactions

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

7.3.8 Serious Adverse Reaction (SAR)

A serious adverse reaction (SAR) is defined as any noxious and unintended response to an IMP related to any dose administered that result in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect. In accordance with ICH-E2A, the definition of an adverse reaction implies a reasonable possibility of a causal relationship between the adverse event and the IMP. An adverse reaction, in contrast to an adverse event, is characterised by the fact that a causal relationship between a medicinal product and an occurrence is suspected. It could also be related to the administration procedure when the procedure is an essential part of the IMP administration.

7.3.9 Suspected Unexpected Serious Adverse Reaction (SUSAR)

Adverse reactions due to medicines administered in a clinical trial that are unexpected and serious.

7.3.10 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)" (see Section 7.3.10). 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.

7.3.11 Expedited Reporting

The Sponsor is responsible for the appropriate expedited reporting of suspected unexpected serious adverse reactions (SUSAR), including special situations reports (i.e. overdose, medication error) and events considered of special interest to the Competent Authorities. The Sponsor will also report all SAEs, including those that are associated to special situation reports (overdose, medication error), which are unlisted/unexpected and related to the trial drug (IMP) to the investigators and to the IEC / IRBs according to current legislation unless otherwise required and documented by the IEC / IRBs. A SUSAR that meets the seriousness criteria of life-threatening and/or results in death must be reported within seven (7) calendar days. A SUSAR that is not life-threatening or does not result in death must be submitted to the regulatory authorities within fifteen (15) calendar days. In addition to the regulatory authorities, a SUSAR may also need to be reported to other recipients including ethics committee and investigators. In the case of a SUSAR which was initially considered to be non-fatal or non-life-threatening, but which turns out to be fatal or life-threatening, as soon as possible and in any event not later than 7 days after the Sponsor became aware of the reaction being fatal or life-threatening.

7.3.12 Assessment of Causal Relationship to the Study Drug

The Investigator must provide an assessment of the causal relationship of each SAE to the clinical trial IMP(s) according to the following criteria:

- **Y (yes):** there is a reasonable possibility that the IMP(s) caused the SAE [(serious adverse reaction (SAR)].
- **N (not):** there is no reasonable possibility that the IMP(s) caused the 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 and no information was shared with the investigator) if none of the above can be used.

7.4 ADVERSE EVENTS REPORTING PROCEDURES

7.4.1 Reporting of Adverse Events

The Sponsor will collect AEs from ICF signature until Day 60 (±3 days) or until the date of death, whichever occurs first. All AEs suspected to be related to the study drug/IMP must be followed-up after the time of therapy discontinuation until the event or its sequelae resolve or stabilize 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 e-CRF. Whenever possible, the Investigator will record the main diagnosis instead of the signs and symptoms normally included in the diagnoses.

Investigators must assess severity (grade) of the event following the NCI-CTCAE v.5 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 a 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 already provided in the e-CRF.

Abnormal laboratory tests occurring during the study should only be recorded in the AE section of the e-CRF if the disorder:

- Is associated with clinically significant symptoms, and/or
- Leads to a 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 a SAE.
- The test result is considered to be clinically relevant by the Investigator.

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

Signs and symptoms: all signs and symptoms present since the diagnosis of SARS-COV-2 infection will be collected, including those related with the underlying disease or other concomitant conditions only if present at the time of ICF signature. A separate focus will be done on targeted COVID-19 signs and symptoms present within the last 24 h prior to screening and listed in Appendix 10. They will be also monitored separately by the research team and collected in a specific form of the CRF.

The "Adverse event form" will be used to report targeted signs and symptoms and medical history in case of any significant change (improvement or worsening) during the study, as well as for events that occur after the first drug infusion or any event related to a study procedure within the study period (according to ICH guidelines).

7.4.2 Reporting of Serious Adverse Events

The Sponsor will collect SAEs from the time of signing of the ICF until Day 60 days (±3 days) or until the date of death, whichever occurs first. Nonetheless, the Sponsor will evaluate any safety information that is spontaneously reported by an Investigator beyond the time frame specified in the protocol.

All SAEs (as defined above) that occur after first study treatment administration 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 e-CRF 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 [redacted], e-mail [redacted] or Pharmacovigilance Department telephone [redacted]

[redacted] for out of office hours (Greenwich Meridian Time [GMT]) assistance on SAE reporting. 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 e-CRF from the investigational staff within one working day.

SAEs occurring during the screening phase (from ICF signature to randomisation) 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, Treatment-related adverse events, and treatment-emergent adverse events of special interest must be followed until the event or its sequelae resolves or stabilizes to at least grade 1, or to an acceptable level according to the Investigator and the Sponsor or his/her designated representative.

7.4.3 Reporting Pregnancy Cases Occurred within the Clinical Trial

National regulations require that clinical trial Sponsors collect information on pregnancies occurring during clinical trials, in which exposure to the IMP(s) 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 occurring within six months from the patient's last plitidepsin administration, or of the female partner of a male patient occurring while the patient is on study drugs or within six months after the last plitidepsin administration, 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 IMP(s) is suspected.
- Possible exposure of a pregnant woman.
- All reports of elevated/questionable or indeterminate beta human chorionic gonadotropins (β-hCGs).

Immediately after detecting a case of suspected pregnancy in a female 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 withdrawal from the trial.

Any pregnancy, suspected pregnancy, or positive pregnancy test must be reported to Pharma Mar S.A. Pharmacovigilance immediately 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 PharmaMar 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 fetal or neonatal death or congenital anomaly) the Investigator will also follow the procedures for reporting SAEs (complete and send the SAE form to Pharma Mar S.A. Pharmacovigilance within 24 hours of the Investigator's knowledge of the event).

All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death at any time thereafter that the Investigator suspects is related to the exposure to the study drug(s)/IMP(s) should also be reported to Pharma Mar S.A. Pharmacovigilance within 24 hours of the Investigators' knowledge of the event.

7.5 ADVERSE EVENTS MONITORING

Safety review will be performed at Pharma Mar, S.A. once the SAE forms have been received and the e-CRFs have been completed by the Investigator.

Periodic safety review of clinical data will be performed; an IDMC will be established to provide study oversight. 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 description of the process will be outlined in the IDMC Charter.

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

Non-serious AEs will be checked for accuracy against the e-CRF during monitoring visits by the monitor.

7.6 PHARMACOKINETICS

7.6.1 Blood Sampling

Blood samples will be collected from the first 30 patients enrolled in this study (experimental arms), at the time points depicted in **Table 3**. In the event that any of the first 30 patients does not complete all three doses, the first 30 patients who complete all 3 doses will be included.

Table 3 Samples for pharmacokinetic evaluations.

Study Day	Sample number	Time point (absolute time from the start of first plitidepsin infusion)	Collection window
1	1	0 h	Before first infusion
3	2	48 h	Before SOI (-20 to -1 m)
	3	49 h	5 m before EOI (±4 m)
	4	52 h	3 h after EOI (±30 m)
	5	55 h	6 h after EOI (±1 h)
4	6	72 h	23 h after EOI (±4 h)
8	7	168 h	119 h after EOI (±24 h)

Abbreviations: EOI=end of third infusion; h, hour; min, minute; SOI=start of third infusion

The infusion rate will be predetermined to ensure that the doses of plitidepsin are infused in 60 min, at a constant rate. In order to obtain reliable PK information, the infusion rate should not be modified once the infusion begins. If a variation in the infusion time eventually occurs, it is very important this to be reflected in the e-CRF. The accurate recording of actual dosing and sampling times is much more important than the strict adherence to the scheduled times.

Blood samples will be obtained into a vacutainer tube by using a peripheral catheter placed in a vein of the arm opposite to the side used for drug infusion. Even the last sample must never be collected from the catheter used for drug infusion.

A Laboratory Manual will be provided with details on collection and storage of PK samples. Please read it carefully before PK sampling. The blood-containing tubes will be stored frozen until their shipment to the Central Laboratory for PK Samples (see details in the Study Contacts). All the material for PK procedures will be provided by the Sponsor).

7.6.2 Analytical Procedures

Blood samples will be analysed to determine concentrations using validated, specific, and sensitive liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS) methods by or under the supervision of the Sponsor.

7.6.3 Pharmacokinetic Parameters

Pharmacokinetic analysis will be the responsibility of the Sponsor in accordance with the current Clinical Pharmacokinetics guidelines on population pharmacokinetic analyses (88, 89). Clearance and volume of distribution will be the primary parameters of interest for the population PK analysis. Additional PK parameters will be calculated, if deemed appropriate.

8. STATISTICAL METHODS

This is an exploratory basket study to adequately approach the heterogeneity of clinical conditions included under the concept of immune compromise.

The study implements a Bayesian methodology. This decision has been adopted after taking into account the relative low frequency of the conditions under study, the relative absence of prospective data of the outcomes of COVID-19 for the study populations (most of the available data are retrospective and belong to the early waves of the pandemic, with different viral variants than nowadays), along with the changing medical practices in these settings. Under these premises, a Bayesian approach is more appropriate in terms of efficient decision-making process, and will provide novel information for future therapeutic development in these clinical conditions (90).

The trial will use a minimally-informative prior of N(0,1) to update the information provided by the trial data to produce a posterior distribution.

The resultant posterior distribution will serve as a starting point for further trials within this area, whether as a key part of any information data packs provided to experts ahead of an elicitation meeting, or directly as the informed prior or hypotheses for further trials (91) (92).

A calibrated Bayesian hierarchical model (CBHM) described by Chu and Yuan (93) will be used to analyse the data, allowing data to be analysed by group and results from each group will be used to enrich the data of the other groups. The calibration of the model allows strong information sharing when the treatment effect is similar among baskets, and shrinks that sharing towards 0 as the treatment effect becomes more heterogeneous.

An overall type I error of 5% was used in analysing by means of credible regions the OR against a null of OR=1 [log(OR)=0], the case where no discernible difference is induced from intervention.

Simulation was used to perform sensitivity analysis on a range of informed priors against a minimally-informative prior to ensure that its use would not impact the results on the data too far.

The statistical methods for this study are described in a detailed statistical analysis plan (SAP).

8.1 SAMPLE SIZE CALCULATION

Our intention is to conduct this study with 4 groups of immunocompromised patients.

A) Randomised groups (1-3)

Patients in three of the four groups will be randomly assigned to one of two arms. Each randomised group will have a sample size of 40 patients (20 in the experimental arm, 20 in the control arm).

Given the exploratory nature of this phase II study, this sample size has been chosen based on operational considerations to allow the feasibility of accrual in this relatively small population and also provide preliminary estimates to establish an updated prior distribution of the mortality rates for further investigation using Bayesian methods in a potential Phase III study.

The operating characteristics of the calibrated Bayesian hierarchical model (CBHM) described by Chu and Yuan (93) have been investigated by means of the CBHM package from Yuan at MD Anderson Cancer Center (94), and with reference to the JAGS program running in R version 4.2.1 (95) on Microsoft Windows 10.

Within the CBHM simulations, 5000 Markov-chain Monte Carlo (MCMC) repetitions per scenario were simulated with a range of operating characteristics for the null, alternative and true mortality rates moving from 5% to 95% in steps of 5% (Table 4). A maximum sample size of 40 per randomised group was set through the CBHM simulations, which in turn determines whether there is a need for the total sample size to be allocated. Within all simulations, the full 120 patients were used, suggesting that this is the minimum sample size that enables decision-making. As an example, if the mortality rate in each group is 20% and we target a clinically meaningful reduction of 12.5% in the experimental arm (H_0=20%, H_1=7.5%), and the assumed true mortality rate is 7.5%, the simulation demonstrates that a sample size of 40 per basket will allow for a posterior probability around 0.9 in each basket (i.e. 90% probability that the

experimental arm is better than the control in each basket), ranging from 0.87 (when the data from the fastest basket is analysed) to 0.915 (at the end of the accrual of the last basket) (type I error rate= 0.05).

Table 4 Example of simulated posterior probabilities for sample size definition.

Mortality rate – control arm (null)	Mortality rate – experimental arm (alternative)	True mortality rate	Sample size per basket	Posterior probability 1st basket	Posterior probability 2nd basket	Posterior probability 3rd basket
20%	7.5%	7.5%	30	0.82	0.84	0.865
20%	7.5%	7.5%	40	0.87	0.88	0.915
20%	7.5%	7.5%	50	0.895	0.905	0.93
20%	7.5%	7.5%	60	0.905	0.925	0.97
20%	7.5%	7.5%	70	0.945	0.98	0.985
20%	7.5%	7.5%	80	0.96	0.985	0.985

The full output of the simulations performed can be found in the Statistical Analysis Plan.

B) Non-controlled group 4*

Many different clinical conditions cannot be classified into one of the 3 randomised groups and have been pooled into a fourth group of 30 patients. As there is a remarkable intragroup heterogeneity, this group will not be controlled and will contribute to the objective of increasing safety knowledge of plitidepsin. A Fleming's phase II procedure with a 1-stage design has been used to estimate the sample size needed to rule out that plitidepsin induces drug-related SAEs in at least 35% of the patients (null hypothesis), and to confirm that the respective rate is 10 % or lower (alternative hypothesis), with a type I error of 0.05 and a power of 90%.

If at least 25 patients out of a total sample of 30 do not experience a drug-related SAE, the null hypothesis will be rejected.

*Not applicable in Spain

8.2 ANALYSIS POPULATIONS

8.2.1 Full Analysis Set (FAS) Population

All randomised patients who have taken at least 1 dose of study treatment (plitidepsin or control) and have completed follow-up for survival until day 30 (±2). Patients who die before the end of follow-up period will also be included in the FAS population. FAS population will be analysed according to their randomised treatment arm.

8.2.2 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 arm.

8.2.3 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

The design of the trial, the Bayesian analysis and the sample size is based on the 30-day (+/- 2 d) survival information on the FAS population. The analysis of the primary and other time-to-event efficacy endpoints will be performed primarily in the FAS but also in the "Per Protocol" and "As treated" populations. Supportive analyses will be performed on the Per Protocol population. Safety endpoints analyses will be based on the "As treated" population.

8.4 ANALYSIS METHODS

8.4.1 Primary Endpoint

The difference between treatment and placebo arms in all-cause mortality at 30 days will be examined using a Bayesian comparison of proportions within the calibrated Bayesian hierarchical model (CBHM) framework described by Chu and Yuan (93). The use of the CBHM allows for flexible information borrowing across all groups, with strong borrowing in the case when treatment effect is homogeneous among the groups and less borrowing as the effect becomes more heterogeneous.

In this framework the odds ratio is modelled as θi=log(oddsi) with a normal distribution of unknown mean (μ) and variance (σ2). The prior distribution used by the framework will be a minimally-informative prior using N(0.1).

The variance (σ2) represents the degree of heterogeneity between the patient groups, with 0 representing the case where there is homogeneity of treatment effect and thus complete pooling of the results across patient groups, with adjustment for targeted p1 rates in each group, whereas a value closer to infinity would lead to no borrowing across groups. These estimates are iteratively updated within the modelling procedure based on the available data.
As each basket completes, they will be evaluated for efficacy, with information gained being used to update the analyses of successive baskets.

Once all patients recruited to a basket have completed the trial, a formal analysis will be performed on the patients within the basket. The result will be able to stand on its own for the

specific subpopulation, and the information from this analysis will be used to enrich the information at analysis of the proceeding baskets.

Results in later baskets will be related to pooled data, however if one basket is not seen to be homogeneous to other baskets, the information from that basket can be run without the pooled information from other baskets and its information not pulled through into later analyses.

As a sensitivity analysis the Bayesian hierarchical model will be used.

The cut-off for considering further clinical development of plitidepsin in each randomised group will be a posterior probability of superiority for the experimental arm equal to or greater than 65%.
Sensitivity/supportive analyses for the primary endpoint will be also performed. Frequentist approach for odds ratio comparison: unstratified and stratified considering randomisation and actual strata values.

8.4.2 Secondary Endpoints/Other Endpoints

Frequency tables will be prepared for categorical variables, and continuous variables will be described by means of summary tables, which will include the median, mean, standard deviation, interquartile range, minimum, and maximum of each variable.
In addition to Bayesian methods, frequentist methods will be also calculated. Counts and percentages, with their corresponding exact confidence intervals, will be calculated for the binomial endpoints. Time-to-event variables and their set time estimates will be analysed according to the Kaplan-Meier method and Cox regressions.
Key secondary endpoint, time to confirmed negativisation in SARS-CoV-2 antigen test or RT-PCR Ct > 30, will be analysed according to the Kaplan-Meier method and Cox regressions following unstratified and stratified test as supportive analyses.

8.4.3 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. 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 will be listed and summarised by system organ class/preferred term.

AEs, SAEs, deaths, laboratory evaluations, and study drug discontinuations due to AEs will be tabulated in a descriptive way. Counts and percentages will be used for categorical variables, and summary tables will be used for continuous variables.

Any 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.

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.

8.4.4 Pooled and Subgroup Analyses

Pooled and subgroup analyses will be performed, taking into consideration baseline characteristics of the patients and of the underlying disease, risks factors for clinical deterioration or death, vaccination status, duration of the infection prior to study entry, and concomitant therapies (e.g., immunomodulatory agents —including glucocorticoids—, oxygen supplementation).
Subgroup analysis of TEAEs and clinically relevant laboratory tests will be performed based on prognostic factors of medical interest.

8.5 SUMMARY OF ESTIMANDS AND STATISTICAL TESTS

The primary estimand corresponding to the primary endpoint is defined as:

Treatment: Participants will be randomised to receive plitidepsin versus control in each group.

Population: Participants as defined through the inclusion and exclusion criteria, who have been randomised to the trial and who have taken at least 1 dose of study treatment (plitidepsin or control).

Variable:

–1-month all-cause mortality rate (Day 30 since randomisation).

Intercurrent events:

–The participant's data are collected and included in the analysis without regard for treatment discontinuation (treatment-policy strategy).

–In the event of the participant initiating another non-protocol therapy, 1-month all-cause mortality rate will be evaluated regardless of initiation of new non-protocol therapy (treatment-policy strategy).

Population level summary: the posterior probability associated for the odds ratio of 1-month all-cause mortality rate from a Bayesian logistic regression for each group comparison.

Below a summary table where main/supportive analyses are described. P values except for the primary analysis have to be considered as exploratory and nominal values will be shown.

Endpoints	**Analyses**
Efficacy primary endpoint	
1-month all-cause mortality rate.	-Bayesian odds ratio (primary analysis by CHBM) -Bayesian odds ratio (Berry's Method) - Unstratified odds ratio (Univariate Logistic regression) - Stratified odds ratio (Multivariate Logistic regression)
Key secondary endpoint	
Time to confirmed negativisation in SARS-CoV-2 antigen test or RT-PCR Ct > 30.	-Kaplan-Meier (unstratified and stratified) -Cox regression (unstratified and stratified)
Efficacy secondary endpoints	
Time to sustained end of COVID-related hospital care from the time of randomisation. Sustained discharge is defined as no subsequent admission within 30 days of initial end of hospital care [up to day 60 (±3)], related to either COVID-19 or COVID-19 therapy	-Kaplan-Meier (unstratified) -Cox regression (unstratified)
Time to sustained improvement (defined in Section 7.1.3) and resolution of selected COVID signs/symptoms (See Appendix 10 - COVID-19 signs/symptoms checklist).	-Kaplan-Meier (unstratified) -Cox regression (unstratified)
Distribution of patients according to their clinical status by the 11-category WHO Clinical Progression Scale, at Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3) (See Appendix 1 – 11-Point World Health Organization (WHO) Clinical Progression Scale).	-Frequencies -Unstratified odds ratio (ordinal)

Endpoints	**Analyses**
Percentage of patients requiring oxygen therapy on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).	-Frequencies -Unstratified odds ratio (ordinal)
Time to sustained discontinuation (i.e., at least 7 days) of oxygen supplementation.	-Kaplan-Meier (unstratified) -Cox regression (unstratified)
Safety secondary endpoints	
Treatment-emergent adverse events (TEAEs).	-Frequencies (severity grade by NCI-CTCAE v5)
TEAEs ≥ grade 3 according to the National Cancer Institute [NCI]-Common Terminology Criteria for AEs (CTCAE v.5.0).	-Frequencies
Adverse events of special interest (AESIs).	-Frequencies (severity grade by NCI-CTCAE v5)
Serious adverse events (SAEs).	-Frequencies (severity grade by NCI-CTCAE v5)
Drug related Serious Adverse Events (i.e., SARs).	-Frequencies (severity grade by NCI-CTCAE v5)
Adverse events leading to treatment discontinuation.	-Frequencies (severity grade by NCI-CTCAE v5)
Deaths (COVID-19-related/all).	-Frequencies
Change respect to baseline in individual study-defined laboratory parameters (See Section 5.7, 5.8 and 5.9 and Appendix 8 - Clinical Laboratory Analyses).	-Frequencies (severity grade by NCI-CTCAE v5)
Change respect to baseline in individual vital signs (See Section 5.7, 5.8 and 5.9).	-Frequencies
Other Secondary/Exploratory Endpoints	
Percentage of patients requiring advanced oxygen support on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2,) and 60 (±3).	-Frequencies -Unstratified odds ratio

Endpoints	**Analyses**
Time to intensification of respiratory support (WHO >5) (See Appendix 1 – 11-Point World Health Organization (WHO) Clinical Progression Scale).	-Kaplan-Meier (unstratified) -Cox regression (unstratified)
Total duration of advanced oxygen support.	-Summary statistics -U-Mann Whitney
Percentage of patients requiring high-flow oxygen on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).	-Frequencies -Unstratified odds ratio
Total duration of high-flow oxygen therapy per patient.	-Summary statistics -U-Mann Whitney
Percentage of patients requiring non-invasive mechanical ventilation on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).	-Frequencies -Unstratified odds ratio
Total duration of non-invasive mechanical ventilation per patient.	-Summary statistics -U-Mann Whitney
Percentage of patients requiring invasive mechanical ventilation or ECMO on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).	-Frequencies -Unstratified odds ratio
Total duration of invasive mechanical ventilation or ECMO per patient.	-Summary statistics -U-Mann Whitney
Percentage of patients requiring admission to ICU on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).	-Frequencies -Unstratified odds ratio
Total duration of intensive care unit (ICU) stay.	-Summary statistics -U-Mann Whitney
Time to onset of additional (i.e., not present at baseline) immune-modulating drugs.	-Kaplan-Meier (unstratified) -Cox regression (unstratified)

Endpoints	**Analyses**
Percentage of patients receiving immune-modulating drugs on Days 1, 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).	-Frequencies -Unstratified odds ratio
Time to onset of additional (i.e., not present at baseline) antiviral drugs.	-Kaplan-Meier (unstratified) -Cox regression (unstratified)
Percentage of patients receiving subsequent antiviral drugs (i.e., not present at baseline) on Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).	-Frequencies -Unstratified odds ratio
Percentage of patients with a new infection by Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).	-Frequencies -Unstratified odds ratio
Cumulative mortality (all-cause and related to COVID-19) by Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).	-Frequencies -Unstratified odds ratio
Percentage of patients in each study group who require subsequent admission within 30 days of initial end of hospital care [up to day 60(±3)], related to either COVID-19 or COVID-19 therapy.	-Frequencies -Unstratified odds ratio
Change respect to Day 1 in the viral load of SARS-CoV-2, measured by RT-PCR, by Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3), in each study group.	-Summary statistics -Univariate test (Fisher exact test or U-Mann Whitney) -Mixed model
Percentage of patients in each study group with undetectable viral load, by Days 4 (±1), 8 (±1), 15 (±1), 30 (±2), and 60 (±3).	-Frequencies -Unstratified odds ratio
Time to either undetectable viral load of SARS-CoV-2 or > 2 logs reduction respect to Day 1.	-Kaplan-Meier (unstratified) -Cox regression (unstratified)

Endpoints	Analyses
Change respect to baseline in inflammatory biomarkers (C- reactive protein [CRP], procalcitonin, lactate dehydrogenase [LDH], ferritin, neutrophil-to-lymphocyte ratio, and multiplex cytokines assay, by Day 4 (±1), 8 (±1), 15 (±1) and 30 (±2) and 60 (±3).	-Univariate test (Fisher exact test or U-Mann Whitney) -Mixed model
Change respect to baseline in individual serological assessments against SARS-CoV-2, by Days 30 (±2), and 60 (±3).	-Univariate test (Fisher exact test or U-Mann Whitney) -Mixed model
Change respect to baseline in individual T-cell response against SARS-CoV-2 by Days 30 (±2), and 60 (±3).	-Univariate test (Fisher exact test or U-Mann Whitney) -Mixed model
Change respect to baseline to Days 15 (±1), and 30 (±2) in chest X-ray findings (Brixia score, centrally assessed) (Appendix 9 – Brixia Score).	-Univariate test (Fisher exact test or U-Mann Whitney) -Mixed model
Percentage of patients requiring modification of the therapy (drugs, dose or schedule) for the underlying disease.	-Frequencies -Unstratified odds ratio
Time in which pre-scheduled therapies for the control of the underlying disease were not able to be administered.	-Kaplan-Meier (unstratified) -Cox regression (unstratified)
Risk for mortality / clinical deterioration according to individual clinical characteristics and laboratory observations present at baseline, scores of validated scales (e.g., ISARIC-4C), as well as features related to COVID-19 [including SARS-CoV-2 variant (if available), vaccination status, duration of the infection, and inflammation-related parameters [neutrophil-to-lymphocyte ratio (NLR), protein C reactive, interleukine-6 (IL-6) and lymphocytes], the underlying disease, and other co-morbidities.	-Frequencies -Unstratified odds ratio
Limited-sampling pharmacokinetics assessment (See Section 7.6).	

Endpoints	Analyses
PK-PD analysis.	

9. ADMINISTRATIVE SECTION

9.1 ETHICS

This clinical trial will be conducted in accordance with the ethical principles that have their origin in the World Medical Association (WMA) Declaration of Helsinki (96) and will be consistent with GCP guidelines and pertinent regulatory requirements.

The study personnel involved in conducting this trial will be qualified by education, training and experience to perform their respective task(s).

The study will be conducted in compliance with the protocol. The protocol, any amendments, and the patient's ICF will receive IEC/IRB approval/favorable opinion prior to initiation. The decision of the IECs/IRBs concerning the conduct of the study will be made in writing to the Investigator and a copy of this decision will be provided to the Sponsor before commencement of the study.

The Investigator and/or the Sponsor is/are responsible for keeping the IEC/IRB informed of any significant new information about the study drug.

All protocol amendments will be agreed upon by the Sponsor and the Investigator.

Administrative changes of the protocol are minor corrections and/or clarifications that have no impact on the way the study is to be conducted.

9.2 MONITORING, AUDITING AND INSPECTING

The study will be monitored by regular site visits and telephone calls to the Investigator by the clinical trial monitor designated by the CRO and supervised by the Sponsor. Source document verification may be performed remotely, if required.

During site visits, the trial monitor should revise original patient records, drug records and document retention (study file). Additionally, the trial monitor should observe study procedures and will discuss any problems with the Investigator.

Adequate time for these visits should be allocated by the Investigator. The Investigator should also ensure that the monitor is given direct access [as per International Conference on Harmonization (ICH) Topic E6 Guideline for Good Clinical Practice, GCP] to source documents (i.e., hospital or private charts, original laboratory records, appointment books, etc.) of the patient which support data entered in the case report forms, as defined in the ICH Topic E6 Guideline for Good Clinical Practice.

Systems and procedures will be implemented to ensure the quality of every aspect of the trial.

At any time during the course or at the end of the study, the Clinical Quality Assurance Department of Pharma Mar S.A. or external auditors contracted by the Sponsor may conduct an onsite or remote audit visit to the centres (ICH-GCP).

Participation in this trial implies acceptance of potential onsite or remote inspection by national or foreign Competent Authorities.

9.3 PATIENT INFORMED CONSENT

The rights, safety and well-being of the trial patients are the most important considerations and should prevail over interests of science and society.

The ICFs will include all elements required by ICH-GCP and applicable regulatory requirements.

Prior to inclusion into the trial, the Investigator or a person designated by the Investigator, must provide the patient with one copy of the ICFs for the clinical trial. This copy must provide written full information about the clinical trial, in a language that is non-technical and easily understood. The Investigator should allow the necessary time for the patient or his/her legally acceptable representative, when required, to inquire about the details of the clinical trial; then, both ICFs must be freely signed and personally dated by the patient and by the person who conducted the Informed Consent discussion before the beginning of the study. The patient should receive a copy of both signed ICFs and any other written information provided to study patients prior to participation in the trial.

During a patient's participation in the trial, any updates to the ICFs and any updates to the written information will be provided to him/her.

If there is a need to obtain new consent from the patients, the Investigator or a person designated by the Investigator should inform the patients of any new information relevant to the patients' willingness to continue participation in the study, before obtaining the written consent.

9.4 CONFIDENTIALITY/ PATIENTS IDENTIFICATION

The collection and processing of personal data from the patients enrolled in this clinical trial will be limited to those data that are necessary to investigate the efficacy, safety, quality and usefulness of the study drug used in this trial.

It is the Investigator's responsibility that sufficient information on the identity of the patients will be retained.

The trial monitor, the Sponsor's auditor, the IECs/IRBs and the Competent Authorities should have direct access to all requested trial-related records, and agree to keep the identity of study patients confidential.

The data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations.

Explicit consent for the processing of personal data will be obtained from the participating patient before data collection, if applicable, and this consent should also address the transfer of the data to other entities and countries.

Pharma Mar, S.A. (as Sponsor´s Headquarter) shall comply with General Data Protection Regulation (GDPR) (EU) 2016/679 effective from 25 May 2018 (repealing Directive 95/46/EC GDPR of 24 October 1995), and applicable regulations on the protection of individuals with regards to the processing of personal data and on the free movement of such data.

9.5 ELECTRONIC CASE REPORT FORMS

Electronic CRFs (e-CRFs) will be used to record all data for each patient. It is the responsibility of the Investigator to ensure that the e-CRFs are properly and completely filled in, in English. E-CRFs must be completed for all patients who have given their informed consent and have been enrolled into the study.

A patient's source documentation is the patient's records (including but not limited to physician/hospital notes, nurses notes, IMP preparation records including reconstitution and dilution, IMP administration records, patient-reported outcomes, etc.) and any original document, and as such they should be maintained at the study site.

The data collected in the e-CRF will be entered into the Sponsor's databases which comply with GDPR (EU) 2016/679 effective from 25 May 2018 (repealing Directive 95/46/EC GDPR of 24 October 1995) on the protection of individuals with regard to the processing of personal data and on the free movement of such data.

9.6 INSURANCE

The Sponsor will provide insurance or indemnity in accordance with the applicable regulatory requirements.

9.7 RETENTION OF RECORDS

The Investigator/Institution should maintain trial documents according to ICH Guideline for Good Clinical Practice and as required by applicable regulatory requirements.

Essential documents should be retained as per the aforementioned ICH guideline or for a longer period of time, if required by the applicable regulations.

9.8 USE OF INFORMATION AND PUBLICATION

Before the investigators of this study submit a paper or abstract for publication or otherwise publicly disclose information concerning the study drug or products, Pharma Mar S.A. must be provided with at least 60 days to revise and approve the proposed publication or disclosure to ensure that confidential and proprietary data are protected.

If Pharma Mar S.A. determines that patentable patient matter is disclosed in the proposed publication or disclosure, the publication or disclosure will be withheld for a period of time considered convenient. If the study is part of a multicentre study, the first publication of the study shall be made in conjunction with the presentation of a joint, multicentre publication of the study results with the investigators and the institutions from all appropriate sites that are contributing data, analysis and comments. However, if such a multicentre publication is not submitted within 12 months after conclusion, abandonment or termination of the study at all sites, the present study may be published individually in accordance with the procedure established above.

The order of the coauthors will reflect the relative contribution of each one to study development and analysis. In general, the first author will be the Investigator who recruits the highest number of patients with information finally available for data analysis. Relevant Pharma

Mar S.A. personnel who have fully participated in the study must be considered for co-authorship of the publication.

10. REFERENCES

1. WHO. Coronavirus disease 2022 [Available from: https://www.who.int/health-topics/coronavirus#tab=tab_1.

2. Mousavizadeh L, Ghasemi S. Genotype and phenotype of COVID-19: Their roles in pathogenesis. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi. 2021;54(2):4.

3. Carotti M, Salaffi F, Sarzi-Puttini P, Agostini A, Borgheresi A, Minorati D, et al. Chest CT features of coronavirus disease 2019 (COVID-19) pneumonia: key points for radiologists. La Radiologia medica. 2020;125(7):10.

4. M. GY, Xu G, Wang B, Liu B-C. Cytokine storm syndrome in coronavirus disease 2019: A narrative review. Journal of internal medicine. 2021;289(2):14.

5. El-Baz LMF, Elwakeel KZ, Elgarahy AM. COVID-19 from mysterious enemy to an environmental detection process: a critical review. Innovative Infrastructure Solutions. 2020;5(3):1-13.

6. P. S, Li W, Xie J, Hou Y, You C. Cytokine storm induced by SARS-CoV-2. Clinica chimica acta; international journal of clinical chemistry. 2020;509:7.

7. WHO Coronavirus (COVID-19) Dashboard 2022 [Available from: https://covid19.who.int.

8. S. KO, Haydel BM, Florman SS, Rana MM, Chaudhry ZS, Ramesh MS, et al. Coronavirus Disease 2019 in Solid Organ Transplant: A Multicenter Cohort Study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2021;73(11):9.

9. A. S, Bhatt NS, St Martin A, Abid MB, Bloomquist J, Chemaly RF, et al. Clinical characteristics and outcomes of COVID-19 in haematopoietic stem-cell transplantation recipients: an observational cohort study. The Lancet Haematology. 2021;8(3):8.

10. K. AR, Chiang TP, Marr KA, Brennan DC, Sait AS, Garibaldi BT, et al. Inpatient COVID-19 outcomes in solid organ transplant recipients compared to non-solid organ transplant patients: A retrospective cohort. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2021;21(7):10.

11. M. SJ, Friman G, von Zur-Mühlen B, Ericzon BG, Wallquist C, Karason K, et al. COVID-19 in solid organ transplant recipients: A national cohort study from Sweden. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2021;21(8):11.

12. M. F, Babik JM. COVID-19 in Immunocompromised Hosts: What We Know So Far. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2021;72(2):10.

13. M. TJ, Swain CE, Pierce JL, Zamboni L, Wu M, Holtgrave DR, et al. COVID-19 Outcomes Among Persons Living With or Without Diagnosed HIV Infection in New York State. JAMA network open. 2021;4(2).

14. S. SK, Tagliamento M, Lambertini M, McNally R, Romano M, Leone M, et al. Mortality in patients with cancer and coronavirus disease 2019: A systematic review and pooled analysis of 52 studies. European journal of cancer (Oxford, England : 1990). 2020;139:7.

15. G. GV, Papoutsi E, Siempos II. Effect of Cancer on Clinical Outcomes of Patients With COVID-19: A Meta-Analysis of Patient Data. JCO global oncology. 2020;6:9.

16. N. S, Bates B, Song Q, Madhira V, Yan Y, Dong S, et al. Outcomes of COVID-19 in Patients With Cancer: Report From the National COVID Cohort Collaborative (N3C). Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2021;39(20):14.

17. R. MA, Roeker LE, Lamanna N, Allan JN, Leslie L, Pagel JM, et al. Outcomes of COVID-19 in patients with CLL: a multicenter international experience. Blood. 2020;136(10):9.

18. M. OD, Ribeiro Dos Santos G, Wang L, Cummings DAT, Azman AS, Paireau J, et al. Age-specific mortality and immunity patterns of SARS-CoV-2. Nature. 2021;590(7844):5.

19. V. B, Ferrarini I, Dell'Eva M, Sbisà E, Krampera M, Visco C. COVID-19 (SARS-CoV-2 infection) in lymphoma patients: A review. World journal of virology. 2021;10(6):13.

20. R. P, Lee SA, Kim SY, de Melo AC, Kasi. Association of active oncologic treatment and risk of death in cancer patients with COVID-19: a systematic review and meta-analysis of patient data. Acta oncologica (Stockholm, Sweden). 2021;60(1).

21. Giné E, Gomes da Silva M, K. TM, Visco C, Kinda S, Hermine O, et al. Outcome of COVID-19 in Patients With Mantle Cell Lymphoma-Report From the European MCL Registry. HemaSphere. 2022;6(5).

22. F. D, Rampotas A, Vallance G, Panitsas F, Basker N, Sangha G, et al. Infections in relapsed myeloma patients treated with isatuximab plus pomalidomide and dexamethasone during the COVID-19 pandemic: Initial results of a UK-wide real-world study. Hematology (Amsterdam, Netherlands). 2022;27(1):8.

23. O. B, Salmanton-García J, Nowak P, Itri F, Van Doesum J, López-García A, et al. Outcome of infection with omicron SARS-CoV-2 variant in patients with hematological malignancies: An EPICOVIDEHA survey report. American journal of hematology. 2022.

24. L. P, Salmanton-García J, Marchesi F, Busca A, Corradini P, Hoenigl M, et al. COVID-19 infection in adult patients with hematological malignancies: a European Hematology Association Survey (EPICOVIDEHA). Journal of hematology & oncology. 2021;14(1).

25. T. N, Ferguson NM, Nash SG, Webster HH, Flaxman S, Andrews N, et al. Comparative analysis of the risks of hospitalisation and death associated with SARS-CoV-2 omicron (B.1.1.529) and delta (B.1.617.2) variants in England: a cohort study. Lancet (London, England). 2022;399(10332):9.

26. A. CP, Olsen RJ, Long SW, Snehal R, Davis JJ, Ojeda Saavedra M, et al. Signals of Significantly Increased Vaccine Breakthrough, Decreased Hospitalization Rates, and Less Severe Disease in Patients with Coronavirus Disease 2019 Caused by the Omicron Variant of Severe Acute Respiratory Syndrome Coronavirus 2 in Houston, Texas. The American journal of pathology. 2022;192(4):10.

27. U. NC, da Cunha-Bang C, Helleberg M, Ostrowski SR, Brieghel C. Patients with CLL have lower risk of death from COVID-19 in the omicron era. Blood. 2022.

28. Y. GI, Vijenthira A, Betschel SD, Hicks LK, Cheung MC. COVID-19 vaccine response in patients with hematologic malignancy: A systematic review and meta-analysis. American journal of hematology. 2022;97(4):3.

29. C. GD, Sahin TK, Kilickap S, Uckun FM. Antibody Responses to COVID-19 Vaccination in Cancer: A Systematic Review. Frontiers in oncology. 2021;11.

30. A. OT, Lu S, Masel R, Zayac A, Paiva K, Rogers RD, et al. Antibody Response to COVID-19 Vaccination in Adults With Hematologic Malignant Disease. JAMA oncology. 2021;7(11):2.

31. Baidaliuk A, Cuypers L, Planchais C, Mouquet H, Baele G, Mouthon L, et al. Serum neutralization of SARS-CoV-2 Omicron sublineages BA.1 and BA.2 in patients receiving monoclonal antibodies. Nature medicine. 2022.

32. G. H, Mellors JW. Improving the Outcomes of Immunocompromised Patients With Coronavirus Disease 2019. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2021;73(6):4.

33. Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, et al. Dexamethasone in Hospitalized Patients with Covid-19. The New England journal of medicine. 2021;384(8):693-704.

34. Therapeutic Management of Adults with COVID-10. [Available from: https://www.covid19treatmentguidelines.nih.gov/therapeutic-management/.

35. H. BJ, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the Treatment of Covid-19 - Final Report. The New England journal of medicine. 2020;383(19):13.

36. A. M, Nevola R, Sellitto A, Cozzolino D, Romano C, Cuomo G, et al. Remdesivir plus dexamethasone versus dexamethasone alone for the treatment of COVID-19 patients requiring supplemental O2 therapy: a prospective controlled non-randomized study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2022.

37. Peto R, Henao-Restrepo AM, Preziosi MP, Sathiyamoorthy V, Abdool Karim Q, Alejandria MM, et al. Repurposed Antiviral Drugs for Covid-19 - Interim WHO Solidarity Trial Results. The New England journal of medicine. 2021;384(6):14.

38. The Safety of Molnupiravir (EIDD-2801) and Its Effect on Viral Shedding of SARS-CoV-2 (END-COVID) 2022 [Available from: https://clinicaltrials.gov/ct2/show/NCT04405739.

39. K. T, Tzou PL, Kosakovsky Pond SL, Ioannidis JPA, Shafer K. Susceptibility of SARS-CoV-2 Omicron Variants to Therapeutic Monoclonal Antibodies: Systematic Review and Meta-analysis. Microbiology spectrum. 2022.

40. M. WK, Rosales R, Yildiz S, Kehrer T, Miorin L, Moreno E, et al. Plitidepsin has potent preclinical efficacy against SARS-CoV-2 by targeting the host protein eEF1A. Science (New York, NY). 2021;371(6532):5.

41. J. R, Muñoz-Basagoiti J, Perez-Zsolt D, Noguera-Julian M, Paredes R, Mateu L, et al. Identification of Plitidepsin as Potent Inhibitor of SARS-CoV-2-Induced Cytopathic Effect After a Drug Repurposing Screen. Frontiers in pharmacology. 2021;12.

42. F. VJ, Landete P, Lopez-Martin JA, Estrada V, Paredes R, Guisado-Vasco P, et al. Preclinical and randomized phase I studies of plitidepsin in adults hospitalized with COVID-19. Life science alliance. 2022;5(4).

43. K. RA, Thorne LG, Zuliani-Alvarez L, Bouhaddou M, Obernier K, Hiatt J, et al. Host-directed therapies against early-lineage SARS-CoV-2 retain efficacy against B.1.1.7 variant. bioRxiv : the preprint server for biology. 2021.

44. M. S, Tenorio R, Fernández de Castro I, Muñoz-Basagoiti J, Perez-Zsolt D, Raïch-Regué D, et al. Unraveling the antiviral activity of plitidepsin against SARS-CoV-2 by subcellular and morphological analysis. Antiviral research. 2022;200.

45. A. L, Muñoz-Alonso MJ, García C, Sánchez-Murcia PA, Martínez-Leal JF, Domínguez JM, et al. Translation Elongation Factor eEF1A2 is a Novel Anticancer Target for the Marine Natural Product Plitidepsin. Scientific reports. 2016;6.

46. K. MM, Kinzy T. eEF1A: thinking outside the ribosome. The Journal of biological chemistry. 2010;285(28):4.

47. R. M, van Zyl M, Fielding BC. The coronavirus nucleocapsid is a multifunctional protein. Viruses. 2014;6(8):27.

48. Zhou B., J. L, Q. W, X. L, X. L, P. L, et al. The Nucleocapsid Protein of Severe Acute Respiratory Syndrome Coronavirus Inhibits Cell Cytokinesis and Proliferation by Interacting with Translation Elongation Factor 1α. J Virol. 2008;82(14).

49. X. Z, Shi H, Chen J, Shi D, Li C, Feng L. EF1A interacting with nucleocapsid protein of transmissible gastroenteritis coronavirus and plays a role in virus replication. Veterinary microbiology. 2014;172(3-4):5.

50. E. GD, Jang GM, Bouhaddou M, Xu J, Obernier K, White KM, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature. 2020;583(7816):9.

51. A. S-MP, Cortés-Cabrera Á, Gago F. Structural rationale for the cross-resistance of tumor cells bearing the A399V variant of elongation factor eEF1A1 to the structurally unrelated didemnin B, ternatin, nannocystin A and ansatrienin B. Journal of computer-aided molecular design. 2017;31(10):13.

52. Anthoney A, Paz-Ares L, C T. Phase I and pharmacokinetic (PK) study of Aplidin (APL) using a 24-hour weekly schedule. Proc Am Soc Clin Oncol 2000;19:189a.

53. Ciruelos EM, Twelves C, MJ D. Phase I clinical and pharmacokinetic study of the marine compound Aplidin (APL) administered as a 3 hour infusion every 2 weeks. Proc Am Soc Clin Oncol. 2002;21:106a.

54. A. IM, Bowman A, García M, Jodrell D, Martinez M, Pardo B, et al. Phase I clinical and pharmacokinetic study of plitidepsin as a 1-hour weekly intravenous infusion in patients with advanced solid tumors. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008;14(10):7.

55. S. F, Chièze S, Delbaldo C, Ady-Vago N, Guzman C, Lopez-Lazaro L, et al. Phase I and pharmacokinetic study of aplidine, a new marine cyclodepsipeptide in patients with advanced malignancies. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(31):9.

56. A. MJ, Belanger K, Seymour L, Matthews S, Roach J, Dionne J, et al. Phase I study of Aplidine in a dailyx5 one-hour infusion every 3 weeks in patients with solid tumors refractory to standard therapy. A National Cancer Institute of Canada Clinical Trials Group study: NCIC CTG IND 115. Annals of oncology : official journal of the European Society for Medical Oncology. 2006;17(9):7.

57. R. S, Plummer R, Oaknin A, Robinson A, Pardo B, Soto-Matos A, et al. Phase I study of weekly plitidepsin as 1-hour infusion combined with carboplatin in patients with advanced solid tumors or lymphomas. Investigational new drugs. 2011;29(6):7.

58. Gomez-Roca C, Besse-Hammer T, Szyldergemajn S. Phase IB study of plitidepsin (APL) with bevacizumab (BEV) in refractory solid tumor patients (pts) Eur J Cancer. 2010;8.

59. Ocio EM, Mateos MV, Prosper F. Phase I study of plitidepsin in combination with bortezomib and dexamethasone in patients with relapsed and/or refractory multiple myeloma. J Clin Oncol. 2016;34(suppl).

60. P. S, Guillem V, Garcia M, Rivera F, Tabernero J, Cullell M, et al. Phase II randomized study of Plitidepsin (Aplidin), alone or in association with L-carnitine, in patients with unresectable advanced renal cell carcinoma. Marine drugs. 2009;7(1):3.

61. E. B, Droz JP, Paz-Ares L, van Oosterom AT, Cullell-Young M, Schlumberger M. Phase II study of plitidepsin 3-hour infusion every 2 weeks in patients with unresectable advanced medullary thyroid carcinoma. American journal of clinical oncology. 2010;33(1):5.

62. C. P, Hartmann JT, Schmittel A, Bokemeyer C, Schneller F, Keilholz U, et al. Phase II study of plitidepsin in pretreated patients with locally advanced or metastatic non-small cell lung cancer. Lung cancer (Amsterdam, Netherlands). 2008;60(3):6.

63. H. D, Gallardo E, Culine S, Galceran JC, Schöffski P, Droz JP, et al. Phase II study of biweekly plitidepsin as second-line therapy for advanced or metastatic transitional cell carcinoma of the urothelium. Marine drugs. 2009;7(3):12.

64. T. E, Thatcher N, Leyvraz S, Miller WH, Couture F, Lorigan P, et al. Phase II study of weekly plitidepsin as second-line therapy for small cell lung cancer. Lung cancer (Amsterdam, Netherlands). 2009;64(1):5.

65. T. E, Thomas J, Miller WH, Gore M, Wolter P, Kavan P, et al. Phase II study of biweekly plitidepsin as second-line therapy in patients with advanced malignant melanoma. Melanoma research. 2009;19(3).

66. R. P, Lorigan P, Brown E, Zaucha R, Moiseyenko V, Demidov L, et al. Phase I-II study of plitidepsin and dacarbazine as first-line therapy for advanced melanoma. British journal of cancer. 2013;109(6):8.

67. V. R, Caballero D, Fermé C, Zucca E, Arranz R, Briones J, et al. Multicenter phase II study of plitidepsin in patients with relapsed/refractory non-Hodgkin's lymphoma. Haematologica. 2013;98(3):6.

68. Mateos MV, Cibeira MT, Richardson PG. Phase II clinical and pharmacokinetic study of plitidepsin 3-hour infusion every two weeks alone or with dexamethasone in RRMM. . Clinical cancer research: an official journal of the American Association for Cancer Research. 2010;16(12):9.

69. I. S, Ocio EM, Oakervee HE, Greil R, Banh RH, Huang SY, et al. Randomized phase III study (ADMYRE) of plitidepsin in combination with dexamethasone vs. dexamethasone alone in patients with relapsed/refractory multiple myeloma. Annals of hematology. 2019;98(9):11.

70. L. vA, Fudio S, Rosing H, Munt S, Miguel-Lillo B, González I, et al. Pharmacokinetics and excretion of 14 C-Plitidepsin in patients with advanced cancer. Investigational new drugs. 2017;35(5):9.

71. Aguareles J, Villares Fernández P, Carralón González MM, Forné C, Martí Ballesteros EM, Pradillo Fernández V, et al. Outcomes and Clinical Characteristics of the Compassionate Use of Plitidepsin for Immunocompromised Adult Patients with COVID-19. 2023.

72. R. Z, Mele F, Florio LL, Bertolino L, Andini R, Galdo M, et al. Liver injury in remdesivir-treated COVID-19 patients. Hepatology international. 2020;14(5):2.

73. Q. C, Yang M, Liu D, Chen J, Shu D, Xia J, et al. Experimental Treatment with Favipiravir for COVID-19: An Open-Label Control Study. Engineering (Beijing, China). 2020;6(10):6.

74. Zhang S, Asquith B, Szydlo R, Tregoning JS, Pollock KM. Peripheral T cell lymphopenia in COVID-19: potential mechanisms and impact. Immunotherapy Advances. 2022;1(1).

75. A. J, Jafarzadeh S, Nozari P, Mokhtari P, Nemati M. Lymphopenia an important immunological abnormality in patients with COVID-19: Possible mechanisms. Scandinavian journal of immunology. 2021;93(2).

76. L. T, Wang Q, Zhang D, Ding J, Huang Q, Tang YQ, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. Signal transduction and targeted therapy. 2020;5(1).

77. Q. Z, Meng M, Kumar R, Wu Y, Huang J, Deng Y, et al. Lymphopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A systemic review and meta-analysis. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2020;96:4.

78. B. D, Wang C, Tan Y, Chen X, Liu Y, Ning L, et al. Reduction and Functional Exhaustion of T Cells in Patients With Coronavirus Disease 2019 (COVID-19). Frontiers in immunology. 2020;11.

79. Y. ZH, Zhang M, Yang CX, Zhang N, Wang XC, Yang XP, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. Cellular & molecular immunology. 2020;17(5):2.

80. S. TC, Huntley K, Berenger BM, Bristow M, Evans DH, Fonseca K, et al. Prolonged SARS-CoV-2 infection following rituximab treatment: clinical course and response to therapeutic interventions correlated with quantitative viral cultures and cycle threshold values. Antimicrobial resistance and infection control. 2022;11(1).

81. Camprubí D, Gaya A, Marcos MA, Martí-Soler H, Soriano A, Mosquera MDM, et al. Persistent replication of SARS-CoV-2 in a severely immunocompromised patient treated with several courses of remdesivir. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2021;104:2.

82. H. BJ, Smith C, Mirabelli C, Valesano AL, Manthei DM, Bachman MA, et al. Prolonged Severe Acute Respiratory Syndrome Coronavirus 2 Replication in an Immunocompromised Patient. The Journal of infectious diseases. 2021;223(1):4.

83. P. G-V, González-Cortijo L, D'Errico G, Serrera-Alvarez A, Sotres-Fernandez G, García-Coca M, et al. Plitidepsin for the management of a cancer patient infected with SARS-CoV-2 while receiving chemotherapy. Annals of oncology : official journal of the European Society for Medical Oncology. 2021;32(10):2.

84. P. G-V, Carralón-González MM, Aguareles-Gorines J, Martí-Ballesteros EM, Sánchez-Manzano MD, Carnevali-Ruiz D, et al. Plitidepsin as a successful rescue treatment for prolonged viral SARS-CoV-2 replication in a patient with previous anti-CD20 monoclonal antibody-mediated B cell depletion and chronic lymphocytic leukemia. Journal of hematology & oncology. 2022;15(1).

85. Nkf. eGFR Calculator 2022 [Available from: https://www.kidney.org/professionals/kdoqi/gfr_calculator.

86. A. B, R M. COVID-19 outbreak in Italy: experimental chest X-ray scoring system for quantifying and monitoring disease progression. La Radiologia medica. 2020;125(5).

87. Ma, C. HT, Andrus M, Dudeck. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. American journal of infection control. 2008;36(5).

88. Us_Fda. Population Pharmacokinetics | FDA 2022 [Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/population-pharmacokinetics.

89. Agency EM. Reporting the results of population pharmacokinetic analyses 2021 [Available from: https://www.ema.europa.eu/en/reporting-results-population-pharmacokinetic-analyses.

90. G. P, Cro S, Mason A, Phillips R, Cornelius V. Design and analysis features used in small population and rare disease trials: A targeted review. Journal of clinical epidemiology. 2022;144:93-101.

91. V. HL, Whitehead J, Eleftheriou D, Tudur-Smith C, Jones R, Jayne D, et al. Elicitation of expert prior opinion: application to the MYPAN trial in childhood polyarteritis nodosa. PloS one. 2015;10(3):e0120981.

92. N. D, Best N, Montague TH. Better decision making in drug development through adoption of formal prior elicitation. Pharmaceutical statistics. 2018;17(4):301-16.

93. Y. C, Y. Y. A Bayesian basket trial design using a calibrated Bayesian hierarchical model. Clinical trials (London, England). 2018;15(2).

94. Ying Yuan 2022 [Available from: https://odin.mdacc.tmc.edu/~yyuan/.

95. Bayesian Graphical Models using MCMC https://cran.r-project.org â€ º web â€ º packages â€ º rjags - Buscar con Google 2022 [Available from: https://www.google.com/search?q=Bayesian+Graphical+Models+using+MCMC+https%3A%2F%2Fcran.r-project.org+%E2%80%BA+web+%E2%80%BA+packages+%E2%80%BA+rjags&oq=Bayesian+Graphical+Models+using+MCMC+https%3A%2F%2Fcran.r-

project.org+%E2%80%BA+web+%E2%80%BA+packages+%E2%80%BA+rjags&aqs=edge..69i57.542j0j4&sourceid=chrome&ie=UTF-8.

96. WMA - The World Medical Association-Declaration of Helsinki 2022 [Available from: https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/.