Extension Study in a Cohort of Adult Patients With SARS-CoV-2 Infection Requiring Hospital Admission and Received Treatment With Plitidepsin in the APLICOV-PC Study

Statistical Analysis Plan

NCT05121740

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E-APLICOV-PC (AV-APL-A-003-21)

Extension study in a cohort of adult patients with SARS-CoV-2 infection requiring hospital admission who received treatment with plitidepsin in the APLICOV-PC.

STATISTICAL ANALYSIS PLAN

Version: 2.0
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-Confidential

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1 Introduction

This statistical analysis plan describes the protocol-defined final analysis that will be performed in the E-APLICOV-PC study (AV-APL-A-003-21).

Abbreviations:

Abbreviation	Term
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATC	Anatomical therapeutic chemical
BMI	Body mass index
bpm	Beats per minute
BSA	Body surface area
ºC	Degrees Celsius
cm	Centimeter/s
COVID-19	Coronavirus disease 2019
CPK	Creatine phosphokinase
DBP	Diastolic blood pressure
ECG	Electrocardiogram
ECHO	Echocardiogram
eCRF	Electronic case report form
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
ICH	International conference on harmonization
G1-X	Grade 1-X
GGT	Gamma-glutamyl transferase
HR	Heart rate
ICU	Intensive care unit
id.	Identifier
i.e.	That is, from Latin *id est*
IgG	Immunoglobulin G
Kg	Kilogram/s
LDH	Lactate dehydrogenase
MedDRA	Medical dictionary for regulatory activities
m	Meter/s
mg	Milligram/s
mmHg	Millimeter of mercury

mMRC	Modified medical research council
ms	milliseconds
MUGA	Multigated acquisition scan
6MWT	Six-minute walk test
N	Number
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Events
PaFi (PAO_2/FiO_2) Ratio	Ratio of partial pressure arterial oxygen and fraction of inspired oxygen
PCR	Polymerase chain reaction
PR	PR interval
PT	Preferred term
Q1	First quartile
Q3	Third quartile
QT	QT interval
RR	Respiratory rate
SAE	Serious adverse event
SaO_2	Oxygen saturation
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SAS	Statistical analysis system
SAEs	Serious adverse events
SBP	Systolic blood pressure
SOC	System organ classes
Std	Standard deviation
ULN	Upper limit of normal
WHO	World health organization
WBC	White blood cell

2 Synopsis

2.1 Title of the study

Extension study in a cohort of adult patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection requiring hospital admission who received treatment with plitidepsin in the APLICOV-PC.

2.2 Justification of the study

The APLICOV-PC proof of concept study demonstrated the antiviral activity of plitidepsin in terms of viral load reduction, recovery induction, lymphocyte and other inflammatory parameters reconstitution.

Approximately 10 to 25% of patients with COVID-19 experience symptoms beyond 3 months, resulting in a significant restriction in their everyday lives due to the sequelae of the disease that may persist for a year or more and entail long-term medical leaves.

The aim of this study is to assess whether the treatment with plitidepsin, by attaining a reduction in the viral load and a faster recovery of the patient, could have a relevant impact in preventing the appearance of sequelae resulting from the SARS-CoV-2 infection.

2.3 Code of the study

E-APLICOV-PC (AV-APL-A-003-21).

2.4 Sponsor

Pharma Mar, S.A

2.5 Design

Multi-site extension study of the APLICOV-PC clinical study follow-up.

To participate in the study, the patients must have previously participated in the APLICOV-PC proof of concept study and may have received:

- 1.5 mg of plitidepsin administered as a 1.5-hour infusion, once a day for 3 consecutive days (total dose 4.5 mg).
- 2.0 mg of plitidepsin administered as a 1.5-hour infusion, once a day for 3 consecutive days (total dose 6.0 mg).
- 2.5 mg of plitidepsin administered as a 1.5-hour infusion, once a day for 3 consecutive days (total dose 7.5 mg).

Patients were enrolled after signing informed consent to participate in this extension study.

2.6 Study Objectives

To evaluate the incidence of post-COVID-19 morbidity and characterize the profile of complications in patients who participated in the APLICOV-PC study.

2.7 Endpoints

Incidence of post-COVID-19 complications.

2.8 Study population

The main objective of the study is to evaluate the incidence of post-COVID-19 morbidity and

characterize the profile of complications in patients who participated in the APLICOV-PC study.

The maximum number of patients who can participate in the trial is limited to 42.

2.9 Inclusion criteria

Patient who participated in the APLICOV-PC study treated with plitidepsin and who gives their consent.

2.10 Exclusion criteria

There are no exclusion criteria for this study.

3 General principles

Planned analyses in this document will be carried out once the database is declared cleaned, closed and approved by Pharma Mar according to the premises described in the Data Management Plan version 1.0 approved on 12-Jan-2022.

Statistical analyses will be performed using Statistical Analysis System (SAS) software version 9.4 and RStudio. The results of the analyses will be presented in editable format (i.e. rtf).

The data will be provided with one decimal place in general. In those cases where greater precision is required, as many decimal places as necessary will be provided.

Before performing the analysis, the following terms should be coded/reviewed and approved:

- Adverse events (AEs), hospital readmissions and COVID-19 complications. Coded according to Medical Dictionary for Regulatory Activities (MedDRA) and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v5.
- Medication. Classified according to the WhoDrug dictionary (latest version available).
- Reconciliation between the clinical database and the Pharmacovigilance database of serious adverse events (SAEs) in the study, including the resolution of all discrepancies.

Analysis will be done separately for each arm and the total population.

Continuous variables will be presented through the mean, median, standard deviation, range, first quartile (Q1) and third quartile (Q3). Categorical variables will be displayed through the distribution of frequencies and percentages; percentages with valid data will be presented and missing values will not be taken into account in the percentage calculation.

4 Imputation

There isno strategies/methods to handle missing data.

5 Study analysis populations

5.1 Definition of the study population to be analyzed

All patients who sign an informed consent form and agree to undergo the set of clinical examinations, imaging and laboratory tests specified in section 10 of the protocol, will be analyzed in order to fulfil the objectives of the study.

5.2 Patients disposition

The number and percentage of patients per center will be provided.
All analysis will be performed separately for each arm and a total column will be added.

5.3 End of treatment

There is no end of treatment for this study as no treatment doses are administered.

5.4 End of study

The number and percentage of patients who completed or not the study will be provided. For those patients who finished the study early, the causes of termination will be described.
The cases considered as early termination are:

- Withdrawal of consent
- Follow up completed
- Lost to follow-up
- Death
- Other

All analysis will be performed separately for each arm and a total column will be added.

5.5 Deaths

The number and percentage of patients who died during the study will be provided. Patients who have died due to an AE will be described in more detail, indicating all the variables related to the AEs registered in the electronic case report form (eCRF).
All the analyses will be performed separately for each arm and a total column will be added.

6 Patients description

6.1 Demographic data description. General considerations.

The characteristics of the patients will be described and the unavailable information will be detailed; not available values will not be taken into account in the percentage calculation, only valid percentages will be presented. The analysis will be carried out on the full population and all results will be provided by treatment arm. Aggregated results as well as patient listings will be provided.

6.2 Patients characteristics

Demographic and baseline data:

- Age (years)
- Gender (male/female)
- Ethnic group (Arab; Caucasian; Latin; Asian; Black; Other)
- Weight (kg)
- Height (cm)
- Body surface area (BSA) (m^2)
- Body mass index (BMI) - calculated

In addition to all the basal characteristics used in APLICOV-PC if it necessary.

7 Analysis of the primary endpoint

The main objective of the study is to evaluate the incidence of post-COVID-19 morbidity and characterize the profile of complications in the cohort of patients who participated in the APLICOV-PC study.
The number and percentage of patients who develop complications related to persistent illness, according to the physician, during the period between the end of the APLICOV-PC proof of concept study and the completion of this extension study by the patient, as well as the percentage of patients who present complications will be detailed.

A description of the following endpoints will be presented:

- Number and percentage of patients who required readmission to hospital and causes, since last visit of APLICOV-PC study until the end of this extension study.
- Number and percentage of patients who needed oxygen therapy and duration of the same, since last visit of APLICOV-PC study until the end of this extension study.
- The maximum, minimum, Q1 and Q3, mean, median and standard deviation (Std) of the duration of the oxygen therapy.
- Number and percentage of patients who presented complications (defined in Annex 5 of the protocol).
- Barthel Index. A description with the frequency and percentage of each dependency category, according to the score obtained, will be provided:
    - 0-20: Total dependency
    - 21-60: Severe dependency
    - 61-90: Moderate dependency
    - 91-99: Slight dependency
    - 100: Independence
- The maximum, minimum, Q1 and Q3, mean, median and Std of the parameters related to pulmonary function will be provided: oxygen saturation (SaO_2), ratio of partial pressure arterial oxygen and fraction of inspired oxygen (PAFI), forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), FEV1/FVC and diffusion test.

- The maximum, minimum, Q1 and Q3, mean, median and Std of the results of the Six-minute walk test (6MWT).
- Frequency and percentage of each category of the modified Medical Research Council (mMRC) scale:
    - 0 (Dyspnea only after strenuous exercise)
    - 1 (Dyspnea occurring when hurrying on level ground or walking up a slight incline)
    - 2 (Dyspnea resulting in walking more slowly than people of the same age on level ground or in stopping for breath when walking at own pace on level ground)
    - 3 (Dyspnea resulting in stopping for breath after walking about 100 meters of after a few minutes on level ground)
    - 4 (Dyspnea severe enough to prevent the person from leaving the house or occurring when dressing or undressing)
- In patients who present pneumonia upon entry into the APLICOV-PC study, number and percentage of patients with abnormalities in the chest X-ray and description of these abnormalities.
- Number and percentage of patients who presented abnormalities ≥G2 in the analytical parameters.
- Number and percentage of patients who presented abnormalities in the electrocardiogram (ECG) and description of these abnormalities.
- In patients suffering a relevant cardiac event during their participation in the APLICOV-PC study, number and percentage of patients who presented abnormalities in the heart Ultrasound/multigated acquisition scan (MUGA) and description of these abnormalities.
- Multivariate analysis to evaluate the relationship of the main covariates in the previous study (APLICOV-PC). More relevant exploratory covariates from the univariate analyses (p-value < 0.10) will be included in the multivariate analyses. The endpoints listed were correlated with the patients' basal characteristics, the degree of severity of the illness, comorbidities, the plitidepsin dosage, the viral load and the clinical evolution documented in the APLICOV-PC study.

8 Other analysis

- The maximum, minimum, Q1 and Q3, mean, median and Std of the vital signs will be provided: systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), temperature and respiratory rate.
- The maximum, minimum, Q1 and Q3, mean, median and Std of ECG parameters.
- Number and percentage of patients with abnormalities in the physical examination. The result will be listed.
- Number and percentage of patients with abnormalities in the neurological examination. The result will be listed.
- A description will be provided with the frequency and percentage of each category of the lung diffusion test:
    - Normal (between the 80 % and 120 % of the reference value)
    - Low (below the 80 % of the reference value)

    - High (over the 120 % of the reference value)
- Number and percentage of patients who have been diagnosed of COVID-19 since the completion of APLICOV-PC study.
- Number and percentage of patients who have received any COVID-19 vaccine and the number of doses.
- Number and percentage of related or unknown post-COVID-19 complication. Worst per patient.
- Number and percentage of all post-COVID-19 complications. Worst grade per patient.
- Number and percentage of related post-COVID-19 complications. Worst grade per patient.
- Number and percentage of patients with at least AE.
- Number and percentage of patients with at least SAE.
- Number and percentage of patients with at least grade ≥ 3 AE.
- Number and percentage of patients with AEs by system organ classes (SOC) and preferred term (PT), regardless of relationship.
- The maximum, minimum, Q1 and Q3, mean, median and Std of laboratory parameters.
- Aplicov-pc summay of Demographic and Other Baseline Characteristics table of patients include in this study by dose and severity of disease.
    - Median (range) time from symptom onset to first plitidepsin administration, days
    - Number of comorbidities, n (%) (none, one, two or more)
    - Comorbidities, n (%) (Cardiac disease, Kidney disease, Liver disease, Lung disease (COPD), Asthma, Diabetes, Hypertension, Obesity)
    - Clinical status at randomization, n (%)
    - Vital signs, median (range) (Body temperature, ºC, Systolic blood pressure, mmHg,
    - Diastolic blood pressure, mmHg, Heart rate, bpm, Respiratory rate, bpm, SpO2
    - B, %, On oxygen at baseline, n (%))
    - Laboratory parameters, median (range) (WBC, x109/L, Platelet count, x109/L, Lymphocytes, x109/L, Serum creatinine, µmol/L, ALT x ULN, AST x ULN, LDH x ULN, CPK x ULN, GGT x ULN, C-reactive protein, Ferritin, D-dimer)
    - log10 copies/mL viral load, median (range)
    - Days from Plitidepsin to first complication
    - Days from hospital discharge to first complication
    - Time from Plitidipesin first-dose to E-APLICOV inclusion (Month)

9 Listing

- Post-COVID-19 complications will be listed.
- Hospital readmission will be listed
- Oxygen therapy will be listed
- The medication administered before starting the 6MWT will be listed.
- The results of the ECG will be listed.

- The results of the chest X-ray will be listed.
- The ECHO/MUGA results will be listed
- The hematological laboratory parameters will be listed.
- The coagulation laboratory parameters will be listed.
- The biochemistry laboratory parameters will be listed.
- The pharmacological treatments will be listed
- The AEs will be listed
- Physical examination will be listed
- The neurological examination will be listed
- The vaccination schedule will be listed
- The COVID-19 (PCR) will be listed
- The COVID-19 history will be listed
- The serology SARS-CoV-2 will be listed

Appendix I

10 Study population

Table 10.1 Analyzed patients

	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
Analyzed patients				

Table 10.2 Subject Disposition by site

Sites	Dose Cohort							
	1.5 mg N=X		2.0 mg N=X		2.5 mg N=X		Total N=X	
	n (%)							
	Recruited	Analyzed	Recruited	Analyzed	Recruited	Analyzed	Recruited	Analyzed
Site 1								
Site 2								
Site …								

Table 10.3 End of study

	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
End of study reasons				
Follow-up completed as per protocol				
Lost to follow-up				
Withdrawal of consent				
Death				
Other				

Table 10.4 Demographics (categorical)

	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
Gender				
Ethnic group				

Table 10.5 Demographics (Continuous)

Continuous endpoints	Dose Cohort																	
	1.5						…						Total					
	N	Mean	Median	Range	Q1	…*	…	…	…	…	…	…	N	Mean	Median	Range	Q1	…*
Age																		
Weight (kg)																		
Height (cm)																		
BSA (m^2)																		
BMI																		

* Q3 and Std.

Appendix II

11 Primary endpoints

Table 11.1.1 End points I by dose cohort

Endpoints	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
Patients who presented post-COVID-19 complications (related or unknown relationship to study disease)				
Patients who presented post-COVID-19 complications (regardless relationship)				
Patients who presented post-COVID-19 complications (related to study disease)				
Patients who required readmission to hospital (regardless of relationship)				
Patients who required readmission to hospital (COVID-19)				
Patients who required readmission to hospital (post COVID-19 complication)				
Patients who required oxygen therapy				
Patients with abnormalities in the chest X-ray*				
Patients who presented abnormalities ≥G2 in the analytical parameters				
Patients who presented abnormalities in the ECG				
Patients who presented abnormalities in the heart Ultrasound/MUGA**				

*Only in patients who present pneumonia upon entry into the APLICOV-PC
** Only in patients who suffered a relevant cardiac event during their participation in the APLICOV-PC study

Table 11.1.2 End points I by Severity of disease

Endpoints	Severity of disease			
	Mild N=X	Moderate N=X	Severe N=X	Total N=X
	n (%)			

Patients who presented post-COVID-19 complications (related or unknown relationship to study disease)				
Patients who presented post-COVID-19 complications (regardless relationship)				
Patients who presented post-COVID-19 complications (related to study disease)				
Patients who required readmission to hospital (regardless of relationship)				
Patients who required readmission to hospital (COVID-19)				
Patients who required readmission to hospital (post COVID-19 complication)				
Patients who required oxygen therapy				
Patients with abnormalities in the chest X-ray*				
Patients who presented abnormalities ≥G2 in the analytical parameters				
Patients who presented abnormalities in the ECG				
Patients who presented abnormalities in the heart Ultrasound/MUGA**				

*Only in patients who present pneumonia upon entry into the APLICOV-PC
** Only in patients who suffered a relevant cardiac event during their participation in the APLICOV-PC study

Table 11.2.1.1 Summary of post-COVID-19 complications, regardless of relationship, by dose cohort and overall. Worst per patient

Post-COVID-19 complications	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
Asthenia				
General discomfort				
….				

Table 11.2.1.2 Summary of post-COVID-19 complications, regardless of relationship, by severity of disease and overall. Worst per patient

Post-COVID-19 complications	Severity of disease			
	Mild N=X	Moderate N=X	Severe N=X	Total N=X
	n (%)			

Anxiety				
Asthenia				
….				

Table 11.2.2 Summary of Complications, Related to study disease, by dose cohort and overall

Post-COVID-19 complications	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
Asthenia				
General discomfort				
….				

Table 11.3.1 Summary of post-COVID-19 complications, by grade and overall. Worst grade per patient. (Dose 1.5 /2 /2.5 mg and total)

		G1		G2		G3		….		Total	
		N	%	N	%	N	%	N	%	N	%
Post-COVID-19 complications	Asthenia										
	…										
	…										
	….										

Table 11.3.2 Summary of post-COVID-19 complications, related to study disease, by grade and overall. Worst grade per patient. (Dose 1.5 /2 /2.5 mg and total)

		G1		G2		G3		….		Total	
		N	%	N	%	N	%	N	%	N	%
Post-COVID-19 complications	Asthenia										
	…										
	…										
	….										

Listing 11.1.1 Post-COVID-19 complications

Dose cohort	Subject id.	Post-COVID-19 complication	Start date	End date	Ongoing	Duration.	First Date Plitidepsin dose	Relationship	Severity of disease

Listing 11.1.2 Post-COVID-19 complications (related to study disease)

Dose cohort	Subject id.	Post-COVID-19 complication	Start date	End date	Ongoing	Duration.	First Date Plitidepsin dose	Relationship	Severity of disease

Table 11.4 Post-COVID-19 complications duration

Continuous endpoints	Dose Cohort																	
	1.5						…							Total					
	N	Mean	Median	Range	Q1	…*	…	…	…	…	…	…	N	Mean	Median	Range	Q1	…*
Post-COVID-19 complications duration																		
Asthenia																		
…																		

* Q3 and Std.

Listing 11.2 Hospital readmission

Dose cohort	Subject id.	Hospital readmission	Reason	ICU admission	Start date	End date	Ongoing	Duration

Table 11.5 Oxygen therapy duration

Continuous endpoints	Dose Cohort																	
	1.5						…							Total				
	N	Mean	Median	Range	Q1	…*	…	…	…	…	…	…	N	Mean	Median	Range	Q1	…*
Oxygen therapy **duration**																		
Type**																		

* Q3 and Std.
**Nasal cannula; Simple face mask; Venturi mask; Non-rebreather mask; Invasive mechanical ventilation (IMV); Other, specify

Listing 11.3 Oxygen therapy required

Dose	Subject id.	Oxygen therapy required	Type	Start date	End date	Ongoing	Duration

Table 11.6 Pulmonary function assessment and diffusion test

Continuous endpoints	Dose Cohort																	
	1.5						…						Total					
	N	Mean	Median	Range	Q1	… *	…	…	…	…	…	…	N	Mean	Median	Range	Q1	… *
Pulmonary function assessment																		
SaO_2 (%)																		
PAO_2/FiO_2																		
Spirometry																		
FVC (ml)																		
FEV1(ml)																		
FEV1 /FVC																		
Lung diffusion reference theoretical value																		

* Q3 and Std.

Table 11.7 Pulmonary function assessment

	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
Spirometry				
Normal (≥80%)				
Reduced (<80%)				
Lung diffusion testing				
Normal				
Low				
High				

Dyspnea assessment (mMRC)				
0				
1				
2				
3				
4				

Table 11.8 Barthel scale

	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
Barthel scale				
Total dependency (0-20)				
Severe dependency (21-60)				
Moderate dependency (61-90)				
Slight dependency (91-99)				
Independence (100)				

Table 11.9 Six-minute walk test (I)

Continuous endpoints- 6MWT	Dose Cohort																	
	1.5						…						Total					
	N	Mean	Median	Range	Q1	…*	…	…	…	…	…	…	N	Mean	Median	Range	Q1	…*
Pre-TEST																		
Weigth (Kg)																		
SBP (mmHg)																		
DBP (mmHg)																		
SpO_2 (%)																		
Heart rate (bpm)																		
Post-TEST																		
SpO_2 (%)																		
Heart rate (bpm)																		
Results																		

Continuous endpoints- 6MWT	Dose Cohort																	
	1.5						…						Total					
	N	Mean	Median	Range	Q1	…*	…	…	…	…	…	…	N	Mean	Median	Range	Q1	…*
Total distance (m)																		
Expected distance (m)																		
Expected percentage (m)																		
Nº laps																		

* Q3 and Std.

Table 11.10.1 Six-minute walk test (II) by dose cohort

Categorical Endpoints (6MWT)	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
Pre-TEST				
Borg scale of dyspnea and fatigue at basal (1):				
Post-TEST				
Borg scale of dyspnea and fatigue after test (1):				
Results				
Stopped or paused				
Other symptoms at the end of the exercise (2)				

(1) 0 (None); 0.5 (Very, very slight), 1 (Very slight); 2 (Slight); 3 (Moderate); 4 (Somewhat severe); 5 (Severe); 6 (Severe); 7 (Very severe); 8 (Very severe); 9 (Very very severe); 10 (Maximal).

(2) Angina; Dizziness; Pain in hip, leg or calf; None

Table 11.10.2 Six-minute walk test (II) by severity of disease

Categorical Endpoints (6MWT)	Severity of disease			
	Mild N=X	Moderate N=X	Severe N=X	Total N=X
	n (%)			
Pre-TEST				
Borg scale of dyspnea and fatigue at basal (1):				
Post-TEST				
Borg scale of dyspnea and fatigue after test (1):				
Results				
Stopped or paused				

Other symptoms at the end of the exercise (2)				

(1) 0 (None); 0.5 (Very, very slight), 1 (Very slight); 2 (Slight); 3 (Moderate); 4 (Somewhat severe); 5 (Severe); 6 (Severe); 7 (Very severe); 8 (Very severe); 9 (Very very severe); 10 (Maximal).
(2) Angina; Dizziness; Pain in hip, leg or calf; None

Listing 11.4 Abnormalities in the chest X-ray

Dose cohort	Subject id.	Date	Radiography result	Specify	Other

Listing 11.5 ECG Abnormalities

Dose cohort	Subject id.	Date	ECG Result	Specify	QT	PR	RR

Listing 11.6 ECHO/MUGA results

Dose cohort	Subject id.	Date	Result	Specify

Listing 11.7 Six-minute walk test results

Dose cohort	Subject id.	Date	Total distance	Number of laps	Stopped or paused	Reason for stopping or pausing

Listing 11.8 Six-minute walk test previous medication

Dose cohort	Subject id.	Date	Active ingredient	Admin. hour	Admin. minuts	Dose

Listing 11.9 Six-minute walk test. Supplementary oxygen during the test

Dose cohort	Subject id.	Supplementary oxygen needed?	Flow type	Flow type specify	Flow

Table 11.11 Patients with abnormalities in the chest X-ray (all patients)

	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
Patients with abnormalities in the chest X-ray				

Table 11.12 Patients who presented abnormalities in the heart Ultrasound/MUGA (all patients)

	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
Patients who presented abnormalities in the heart Ultrasound/MUGA				

Table 11.13 Multivariable analysis

Variable	Value	DF	Estimate	Standard error	Wald Chi-Square	Pr > Chi-Square	Effect	Odds Ratio Estimate	Lower 95% Confidence Limit for Odds Ratio	Upper 95% Confidence Limit for Odds Ratio

12 Other analysis

Table 12.1 Vital signs

Continuous endpoints	Dose Cohort																	
	1.5						…						Total					
	N	Mean	Median	Range	Q1	…*	…	…	…	…	…	…						
SBP (mmHg)																		
DBP (mmHg)																		
Heart rate (bpm)																		
Temperature (ºC)																		
Respiratory rate (breaths/min)																		

* Q3 and Std.

Table 12.2 Physical and neurological examination

Endpoints	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
Physical examination				
Normal				
Abnormal				
Neurological examination				
Normal				
Abnormal				

Listing 12.1 Physical examination

Dose cohort	Subject id.	Physical examination	Date	System	Finding

Listing 12.2 Neurological examination

Dose cohort	Subject id.	Neurological examination	Date	Finding

Table 12.3 Electrocardiogram

ECG Continuous endpoints	Dose Cohort																	
	1.5						…						Total					
	N	Mean	Median	Range	Q1	…*	…	…	…	…	…	…	N	Mean	Median	Range	Q1	…*
QT (ms)																		
PR (ms)																		
RR (ms)																		

* Q3 and Std.

Table 12.4 COVID-19 (PCR)

COVID-19 (PCR)	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
Has a PCR test been performed?				
Yes				
No				
PCR result				
Positive				
Negative				

Listing 12.3 COVID-19 (PCR)

Dose cohort	Subject id.	PCR test been performed	Date	Result

Table 12.5 COVID-19 (history and IgG serology)

COVID-19	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
Has the patient been diagnosed of COVID-19 since the completion of APLICOV-PC study?				
Yes				
No				
Results IgG				
Positive				
Negative				
Inconclusive				
Has the patient received any COVID-19 vaccine?				
Yes				
No				
Doses received				
1				
2				
3				

Listing 12.4 COVID-19 history

Dose cohort	Subject id.	Diagnosed of COVID-19 since the completion APLICOV-PC study	Diagnosed date	End infection Date	Ongoing

Listing 12.5 COVID-19 Serology SARS-CoV-2

Dose cohort	Subject id.	Serology performed	Date	Result IgG

Listing 12.6 Vaccination schedule

Dose cohort	Subject id.	Vaccination	Dose	Vaccine type	Date

Table 12.6 Pharmacological treatment

Pharmacological treatment		1.5 mg N=X		2.0 mg N=X		2.5 mg N=X		Total N=X	
		N	%	N	%	N	%	N	%
Has the patient received any pharmacological treatment?									
ATC1	ATC2								
	ATC2								
ATC1	ATC2								

Listing 12.7 Pharmacological treatment

Dose cohort	Subject id.	Active ingredients	Start date	End Date	Ong.	Duration	Dose	…(*)

* Units, Frequency, Administration route and Indication.

Table 12.7 Summary of Adverse Events

Patients	1.5 mg N=X		2.0 mg N=X		2.5 mg N=X		Total N=X	
	N	%	N	%	N	%	N	%
Patients with AEs								
Patients with Grade >=3 AEs								
Patients with SAEs								

Table 12.8 Summary of AEs, regardless of relationship, by SOC and PT by dose cohort and overall

MedDRA SOC Preferred Term	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
	n (%)			
Subjects with any AE				
Gastrointestinal disorders				
Abdominal pain				
….				
General disorders and administration site conditions				
Asthenia				
Chest discomfort				
…				

Each patient reported once per Preferred Term (PT);

Table 12.9 Summary of AEs, regardless of relationship by SOC and PT by grade and overall. Worst grade per patient. (Dose 1.5 /2 /2.5 mg and total)

AEs		G1		G2		G3		….		Total	
		N	%	N	%	N	%	N	%	N	%
Gastrointestinal disorders	Abdominal pain										
	…										
	…										
	….										

Listing 12.8 All adverse events

Dose cohort	Subject id.	Age	SOC	PT	Serious adverse events	Relationship	Grade	Onset date	End date

Table 12.10 APLICOV - Patient description

	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
Age, median (range) years				
Gender, % male				
Median (range) time from symptom onset to first administration, days				
Number of comorbidities, n (%)				
None				
One				
Two or more				
Comorbidities, n (%)				
Cardiac disease				
Kidney disease				
Liver disease				
Lung disease (COPD)				
Asthma				
Diabetes				
Hypertension				
Obesity				
Clinical status at baseline, n (%)				
Mild COVID-19 infection				
Moderate COVID-19 infection				
Severe COVID-19 infection				
Vital signs, median (range)				
Body temperature, °C				
Systolic blood pressure, mmHg				
Diastolic blood pressure, mmHg				
Heart rate, bpm				
Respiratory rate, bpm				
SpO_2 at room air, %				
On oxygen at baseline, n (%)				

	Dose Cohort			
	1.5 mg N=X	2.0 mg N=X	2.5 mg N=X	Total N=X
Laboratory parameters, median (range)				
WBC, $x10^9$/L				
Platelet count, $x10^9$/L				
Lymphocytes, $x10^9$/L				
Serum creatinine, µmol/L				
ALT, x ULN				
AST, x ULN				
LDH, x ULN				
GGT, x ULN				
CPK, x ULN				
Ferritin (ng/mL)				
D-dimer (ng/mL)				
C-reactive protein (mg/L)				
log_{10} viral load, median (range) copies/mL				
Days from Plitidepsin to first complication				
Days from hospital discharge to first complication				
Time from Plitidepsin first-dose to E-APLICOV inclusion (month)				

(*) Further covariates could be added upon request

Table 12.11 APLICOV - Patient description by severity of disease

	Dose Cohort			
	Mild N=X	Moderate N=X	Severe N=X	Total N=X
Age, median (range) years				
Gender, % male				
Median (range) time from symptom onset to first administration, days				
Number of comorbidities, n (%)				
None				
One				
Two or more				
Comorbidities, n (%)				
Cardiac disease				
Kidney disease				
Liver disease				
Lung disease (COPD)				
Asthma				
Diabetes				
Hypertension				
Obesity				
Clinical status at baseline, n (%)				
Mild COVID-19 infection				
Moderate COVID-19 infection				
Severe COVID-19 infection				
Vital signs, median (range)				
Body temperature, °C				
Systolic blood pressure, mmHg				

	Dose Cohort			
	Mild N=X	Moderate N=X	Severe N=X	Total N=X
Diastolic blood pressure, mmHg				
Heart rate, bpm				
Respiratory rate, bpm				
SpO_2 at room air, %				
On oxygen at baseline, n (%)				
Laboratory parameters, median (range)				
WBC, $x10^9$/L				
Platelet count, $x10^9$/L				
Lymphocytes, $x10^9$/L				
Serum creatinine, µmol/L				
ALT, x ULN				
AST, x ULN				
LDH, x ULN				
GGT, x ULN				
CPK, x ULN				
Ferritin (ng/mL)				
D-dimer (ng/mL)				
C-reactive protein (mg/L)				
log_{10} viral load, median (range) copies/mL				
Days from Plitidepsin to first complication				
Days from hospital discharge to first complication				
Time from Plitidepsin first-dose to E-APLICOV inclusion (month)				

Table 12.12 Laboratory parameters

ECG Continuous endpoints	Dose Cohort																	
	1.5						…						Total					
	N	Mean	Median	Range	Q1	…*	…	…	…	…	…	…	N	Mean	Median	Range	Q1	…*
Hematology parameters																		
…																		
Coagulation parameters																		
…																		
Biochemistry parameters																		
…																		

* Q3 and Std.
Hematology: Basophils, Eosinophils, Hematocrit, Hemoglobin, Leukocytes, Lymphocytes, Monocytes, Neutrophils, Platelets.
Biochemistry: ALT, AST, Albumin, BUN, C-Reactive Protein, CPK, Calcium, Creatinine, Ferritin, GGT,

Glomerular filtrate, Glucose, LDH, Magnesium, Phosphatase alkaline, Potassium, Sodium, Total bilirubin, Troponin I, Troponin T.
Coagulation: Activated Partial Thromboplastin, D-Dimer, INR, Prothrombin Time

13 Other Listings

Listing 13.1 Hematological laboratory parameters

Dose	Subject id.	Date	Parameter	Value	Units	…(*)

*Range, grade, result and clinically significant

Listing 13.2 Coagulation laboratory parameters

Dose	Subject id.	Date	Parameter	Value	Units	…(*)

*Range, grade, result and clinically significant

Table 13.3 Biochemical laboratory parameters

Dose	Subject id.	Date	Parameter	Value	Units	…(*)

*Range, grade, result and clinically significant

14 ICH Listings

Following ICH E-3 guideline, patient listings will be performed.

- 16.2.1 Discontinued Patients
- 16.2.2 Protocol Deviations
- 16.2.3 Patients Not Included in the Efficacy Analysis
- 16.2.4 Demographic Data
- 16.2.5 Compliance and/or Drug Concentration Data – Not applicable as data will not be collected
- 16.2.6 Individual Efficacy Response Data - Not applicable as data will not be collected
- 16.2.7 Adverse Event Listing (each patient)
- 16.2.8 Listing of Individual Laboratory Measurements by Patient

15 History of changes

Clarifications and modifications have been added to the SAP v2.0 on date 17 Nov 2022.

All changes are included due to improved medical compression of this study.

The following tables have been added:

- *Table 11.1.2 End points I by Severity of disease*
- *Table 11.2.1.2 Summary of post-COVID-19 complications, regardless of relationship, by severity of disease and overall. Worst per patient*
- *Listing 11.1.2 Post-COVID-19 complications (related to study disease)*
- *Table 11.10.2 Six-minute walk test (II) by severity of disease*
- *Table 12.10 APLICOV - Patient description*
- *Table 12.11 APLICOV - Patient description by severity of disease*
- *Table 12.12 Laboratory parameters*

"I have read this statistical analysis plan and confirm that to the best of my knowledge it accurately describes the analytical methods".

Reviewers:

NAME :
TITLE :

Signature_____
date_____

NAME:
TITLE :

Signature_____
date_____

Approver:

NAME:
TITLE :

Signature_____
date_____