PROTOCOL: 2020-SPMS0485-PD-01

EFFECT OF A FOOD SUPPLEMENT ON PRO-INFLAMMATORY AND PRO-RESOLVING MEDIATORS IN PATIENTS WITH POST COVID-19 CONDITION (CHESOLCOV-19).

Clinical Study Report

DRAFT 1

26 January, 2023

1 TITLE PLAGE

STUDY TITLE: Effect of a food supplement on pro-inflammatory and pro-resolving mediators in patients with Post COVID-19 condition**.**

INVESTIGATIONAL PRODUCT: LIPINOVA R-300

INDICATION STUDIED: Post COVID-19 condition / Long COVID-19

STUDY DESIGN: A pilot, prospective, multicentre, randomized, double blind, with parallel design and placebo controlled clinical trial (Chesolcov-19)

NAME OF THE SPONSOR: Chemo Group

PROTOCOL IDENTIFICATION CODE: 2020-SPMS0485-PD-01

EUDRA-CT: N/A

STUDY INITIATION Date (first subject enrolled): 15/11/2021

STUDY COMPLETION DATE (last subject completed): 05/07/2022

INVESTIGATORS:

- Asunción Gracia Aznar
- Isabel Jimeno Sanz
- Pilar Rodríguez Aedo
- Lorenzo Armenteros del Olmo

This clinical study and the archive of the essential documents have been conducted in accordance with the guide of good clinical practice.

DATE OF THE REPORT: 26 January 2023

2 SYNOPSIS

Name of the Sponsor: Chemo Group **Name of the finished products:** **CAP LIPINOVA** **Name of Active Ingredients:** EPA, DHA, 17-HDHA, 18-HEPE, 14-HDHA	Individual Study Table Referring to Part of the Dossier Volume: Page:	*(For National Authority Use Only)*
Title of Study: Effect of a food supplement on pro-inflammatory and pro-resolving mediators in patients with Post COVID-19 condition.		
Investigators: • Asunción Gracia Aznar • Isabel Jimeno Sanz • Pilar Rodríguez Aedo • Lorenzo Armenteros del Olmo		
Study Centres: • Zaragoza: Centro de Salud Hernán Cortés • Madrid: Centro de Salud Isla de Oza • Lugo: Centro de Salud Illas Canarias/Hospital Universitario Lucus Augusti		
Publication (reference): N/A		
Study Period: Start (first patient enrolled): 15/11/2021 End (Last patient last visit): 05/07/2022		
Objetives: **Primary Objective:** To Study the effect of an omega 3 rich food supplement, on pro-inflammatory and pro-resolving lipid mediators in patients with Post COVID-19 condition. **Secondary endpoints:** • To study the effect of the food supplement on the fatigue and dyspnea • To assess the safety and tolerability of the food supplement.		
Methodology: The study was a pilot, prospective, multi-centre, randomized, double-blind, with 4 branches in a parallel design trial. 3 Branches are placebo controlled		
Number of Subjects (planned and analysed): **Planned**: 53 long COVID-19 patients **Analysed**: 53 long COVID-19 patients		
Diagnosis and main criteria for inclusion: The study was conducted in adult, long COVID-19 patients of either sex. The patients should have a positive COVID-12 test before 12 weeks of their inclusion in the study. Eligible subjects should experience fatigue of dyspnea. Fertile women should have a negative pregnancy test before their inclusion and use a very effective contraceptive method thorough the study.		
Test product, dose and mode of administration, batch number: LIPINOVA R-300 capsules, food supplement rich in omega-3 oil, was administered orally. Batch number: 20006178		
Duration of Treatment: 84 days		
Reference therapy, dose and mode of administration, batch number		

Name of the Sponsor: Chemo Group **Name of the finished products:** **CAP LIPINOVA** **Name of Active Ingredients:** EPA, DHA, 17-HDHA, 18-HEPE, 14-HDHA	Individual Study Table Referring to Part of the Dossier Volume: Page:	*(For National Authority Use Only)*

Placebo capsules administered orally.
Batch number: 2100001

Criteria for Evaluation:

Safety:

- Adverse events (AEs)
- Serious adverse events (SAE)
- Abnormalities in vital signs and physical examination

Efficacy:

- The Fatigue Severity Scale (FSS) evolution
- The mMRC (Modified Medical Research Council) dyspnea scale
- Laboratory analyses. Evolution in the plasma and serum concentration of the following
    - FAtty Acid: EPA, DHA, ARA, DPA
    - Monohydroxilated SPMs: 17-HDHA, 18-HEPE, 14-HDHA
    - Resolvins: RvE1, RvD1, RvD2, RvD3, RvD4, RvD5
    - Maresins: MaR1, MaR2
    - Protectins: PD1, PDX
    - Lipoxins: LXA4, LXB4
    - Prostaglandines: PGE2, PGD2, PGF2α
    - Tromboxanes: TXB2
    - Leukotrienes: LTB4

Statistical Methods:

Quantitative variables were described as their average ±SD or 95% percent confidence intervals. Qualitative variables were described as frequencies and percentages. Changes from the baseline were calculated using the ANOVA test, using multiple testing corrections (or the non-parametrical equivalent if the variable does not follow a normal distribution)

Adverse events and serious adverse events were coded using MedDRA version 25.1, tabulated and sorted by SOC and PT. The incidence of AE in all the groups was calculated.

A contrast of hypothesis was considered significant when the corresponding p-value was less than 0.05.

Summary – Conclusions:

Conclusion:

Date of Report: 20 October 2022

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4 LIST OF ABBREVIATIONS AND DEFINITON OF TERMS

Abbreviation	Definition
14-HDHA	14-hydroxy-docosahexaenoic Acid
17-HDHA	17-hydroxy-docosahexaenoic Acid
18-HEPE	18-hydroxy-eicosapentaenoic Acid
AE	Adverse Event
AEMPS	Agencia Española de Medicamentos y Productos Sanitarios
AFC	Alveolar Fluid Clearance
AR	Adverse Reaction
ARDS	Acute Respiratory Distress Syndrome
CRF	Case Report Form
CRO	Clinical Research Organisation
CSR	Clinical Study Report
DHA	Docosahexaenoic Acid
EC	Ethics Committee
EoS	End of Study
EPA	Eicosapentaenoic Acid
FTH	Fundación Teófilo Hernando
FSS	Fatigue Severity Scale
GCP	Good Clinical Practice
IC	Informed Consent
IP	Investigational Product
ISF	Investigator Site File
ITT	Intention To Treat
LTB4	Leucotrien B4
LX (A4, B4)	Lipoxins
Ma (R1, R2)	Maresins
MedDRA	Medical Dictionary for Regulatory Activities
mMRC	Modified Medical Research Council
PD (1, X)	Protectins
PG (E2, D2, F2α)	Prostaglandins
PI	Principal Investigator
PP	Per Protocol
SAE	Severe Adverse Event
SEMG	Sociedad Española de Médicos Generales y de Familia (Spanish society of general practitioners and family doctors)
SPM	Specialized Pro-resolving lipid Mediator
SOP	Standard Operation Procedure
SS	Safety Set
TXB2	Thromboxane B2

5 ETHICS

5.1 Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

The protocol and informed consent (IC) form for this study were reviewed and approved by the responsible Ethics Committees (EC), Comité de Ética de la Investigación de Santiago-Lugo. The submission to the EC was done by Fundación Teófilo Hernando (FTH). The EC approval of the study was provided to FTH, before subjects were screened for entry. Amendments to the protocol were reviewed and approved in the same manner before being implemented. Details of the Ethics Committee is provided in Appendix 16.1.3.

5.2 Ethical Conduct of the Study

This study was designed and monitored in accordance with the Fundación Teófilo Hernando (FTH), standard operating procedures (SOPs), which comply with the ethical principles of Good Clinical Practice (GCP) as required by the major regulatory authorities, and in accordance with the current Helsinki Declaration (Fortaleza, Brazil, October 2013).

5.3 Subject Information and Consent

Informed consent was obtained from each subject before was admitted to the study. Subjects were informed about the clinical trial by an investigator or co-investigator of the research team who explained the procedures of the study, along with the characteristics of the product under investigation and its possible derivative adverse effects in comprehensible, non-technical terms. The investigator gave the subjects enough time to consider their participation in the study. All subjects had time to talk to the investigator and ask as many questions as they wanted before they decided about their participation in the study. The investigator did not undertake any step specifically required for the clinical study until valid consent had been obtained. The consent form, including the date when it was signed, was retained by the investigator as part of the study records (investigator site file – ISF). A copy of the signed informed consent form was given to the subject. Templates of the subject information and informed consent form are included in Appendix 16.1.3.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The study was planned to be conducted in 3 centres, all located in Spain. A complete list of investigators is provided in Appendix 16.1.4. The signature page to this clinical study report (CSR) is provided in Appendix 16.1.5.

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7 INTRODUCTION

COVID-19 is a novel disease caused by the infection of the coronavirus SARS-CoV-2, which spread extensively through aerosols. Surfaces that have been contaminated with the virus can remain infectious for days after the exposition. The virus can make its wat to the mucus membranes in the mouth and nostrils, even the eye's connective tissue, from those infected surfaces through the contact with the hands. The coronavirus disease (COVID-19) outbreak was first reported in 2019 and rapidly spread through all the world causing the actual pandemic.

The clinical manifestations of the disease are diverse and unspecific, and the symptoms very variable. The infection is usually asymptomatic or show mild to moderate symptoms, however, in some cases the Covid-19 disease can cause severe symptoms, including bilateral pneumonia, lung failure, multi-organic failure and death.

In most cases, COVID-19 patients, feel better within a few days or weeks of the appearance of the first symptoms and make a full recovery within 12 weeks. However, for some people symptoms can persist for weeks or months following the infection. The long-term effects of COVID-19 affects several body systems, including pulmonary, cardiovascular and nervous systems, as well as psychological effects. These effects appear to occur irrespective of the initial severity of infection, even in mild or moderate cases, the infection can cause long term organ damage, but occur more frequently in women, middle age, and in those who initially show more symptoms.

Post COVID-19 condition, also known as long COVID, occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months from the onset of COVID-19. Symptoms last for at least 2 months and cannot be explained by an alternative diagnosis. Symptoms may appear following initial recovery from an acute COVID-19 episode, or persist from the initial SARS-CoV-2 infection. Symptoms may also fluctuate or relapse over time (World Health Organization. 2021).

An exacerbated inflammatory response is recognized as a main component in many chronic diseases, including vascular diseases, metabolic syndromes and neurologic diseases. The acute inflammatory response can be divided into two different process, initiation and resolution, a process that, for many years, was considered passive (Tabas & Glass, 2013). Only after the discovery of the first mediators with pro-resolution capabilities, the processes that lead to the resolution of the acute inflammatory response began to be considered active processes (Serhan et al., 2000, Serhan et al., 2002). The anti-inflammatory proprieties of the Omega-3 fatty acids have been known for a long time. These fatty acids are in competition with the arachidonic acid leading to lower levels of pro-inflammatory eicosanoids. During the resolution process, the Omega-3 fatty acids are used to produce signalling molecules as resolvins, protectins and lipoxins, specialised pro-resolution mediators that are known SPMs. These SPMs are agonists that shorten the resolution of the inflammatory response, via the stimulation of resolution key events, stopping the flow of neutrophils, improving the elimination of the apoptotic cells and bacterial death (Bannenberg et al., 2005; Spite et al., 2009; Chiang et al., 2012).

The pro-resolution actions of these mediators are exemplified by their role in pulmonary inflammation. Resolvins, protectins and lipoxins each have a pro-resolution role in mouse models of allergic airway inflammation and infections both bacterial and viral (Rogerio et al., 2012). Lung damage activates the immune system, which releases pro-inflammatory proteins, increases the neutrophils influx in the alveolar space, and promote the local biosynthesis of pro-resolution lipid mediators such as resolvins, maresins, protectins and lipoxins (Matthay et al., 2012). Recent studies show that SPMs can regulate the Alveolar Fluid Clearance (AFC) in Acute Respiratory Distress Syndrome (ARDS) to protect lung function (Wang et al., 2014).

The food supplement studied is rich in Omega-3 fatty acids. Previous studies have showed that it can raise SPMs in serum and plasma in a variety of physiological and pathological circumstances. During inflammation, caused by a trauma or an infection, there is a deficit of SPMs. It is hypothesized that the administration of this nouvelle formula could improve significantly the SPMs levels both in plasma and serum as well as the ration between the SPMs and inflammatory prostaglandins

Previous studies (Elajami et al., 2016; Souza et al., 2020) used doses that ranged from 1500 mg and 3000 mg. Serhan used the formula for a year, whereas in other studies it was administered for 1 to 5 days. The common grounds for all the studies were

a. The lack of adverse reactions
b. Significant raise of SPMs.

Considering the available data, the use of the food supplement rich in Omega-3 fatty acid will not be related to the onset of adverse reactions and that the expected rise of SPMs will be associated to a clinical improvement on the symptoms of patients with Post COVID-19 condition, which, in turn, could endorse the use of the supplement as an addition for the management of the disease.

The measurement of the plasma and serum concentrations of pro-inflammatory (prostaglandins and Leukotrienes) and pro-resolving lipid mediators (lipoxins, resolvins, protectins, maresins and Monohydroxilated mediators derived from EPA and DHA) in patients with Post COVID-19 condition, provided a very valuable information about the immunological response of the patients regarding the inflammatory condition caused by the infection.

8 STUDY OBJECTIVES

8.1 Primary Objective

The primary objective of the study was to assess the effect of a food supplement rich in omega-3 oils on the concentrations of pro-inflammatory and pro-resolving lipid mediators in patients with Post COVID-19 condition.

8.2 Secondary Objectives

As secondary objectives, the effect of the food supplement on the fatigue and dyspnea on patients with Post COVID-19 condition was evaluated. The safety and tolerability of the product were assessed.

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan – Description

The study was designed as a randomized, double blind, with 4 parallel treatment groups, placebo controlled trial to assess the efficacy of a food supplement with high omega-3 oils content in patients with Post COVID-19 condition. The measurements included the levels of pro-inflammatory and pro-resolution lipid mediators as well as the perceived fatigue and dyspnea measured through subjective questionnaires. The safety and tolerability of the investigational product (IP) was also evaluated.

The study was planned as a proof of concept; it is a pilot study that aimed to determine the effect of increasing doses of the food supplement. Two different doses of the supplement were tested and controlled with placebo. An additional low dose group was added, independent of the other 2, that was not controlled with the same objectives, to test the effect of the supplement on the levels of pro-inflammatory and pro-resolving lipid mediators.

Patients who were willing to participate, signed the informed consent (IC) form and fulfilled all the inclusion criteria (and none of the exclusion criteria), were randomized to one of the 4 treatment options, 3 of which correspond to the double blind placebo controlled trial (A, B, C), and the fourth independent, non-controlled, low dose group (X).

No follow-up phase was planned after the study.

The following procedures were done during each visit of the study.

Screening visit – V0 (Day_{-7}/Day_{-3})

- Anamnesis and physical exam
- Measure the body temperature, Blood pressure and heart rate
- Offer the participation in the study

- Give oral and written information and obtain the informed consent
- Check the inclusion/exclusion criteria
- Review the current concomitant medication

Randomization visit – V1 (Day_1)

This visit took place between 3-7 days after the screening visit.

- Check the inclusion/exclusion criteria
- Physical exam
- Measure the body temperature, Blood pressure and heart rate
- Blood sample
- Pregnancy test (if applicable)
- Fatigue Severity Scale (FSS) test
- Modified Medical Research Council (mMRC) Dyspnea Scale
- Randomization
- Record of Adverse Events (AEs)
- Concomitant medication
- Record of intercurrent or concomitant illness
- Provide the IP(s)
- Provide the patient's diary
- Provide instructions about the completion of the diary

Interim visit – V2 ($Day_{28\pm3}$):

4 weeks after the beginning of the treatment (± 3 days), the patients returned to the centre to attend to the interim visit

- Physical exam
- Measure the body temperature, Blood pressure and heart rate
- Blood sample
- Fatigue Severity Scale (FSS) test
- Modified Medical Research Council (mMRC) Dyspnea Scale
- Record of Adverse Events (AEs)
- Concomitant medication
- Record of intercurrent or concomitant illness
- Return of the empty and unused product containers
- Patient's diary review
- Provide the IP(s)

End of Study visit - V3 ($Day_{84\pm3}$)

12 weeks after the first administration of the IP, the patients returned to the center for the final visit

- Physical exam
- Measure the body temperature, Blood pressure and heart rate
- Blood sample
- Fatigue Severity Scale (FSS) test
- Modified Medical Research Council (mMRC) Dyspnea Scale
- Record of Adverse Events (AEs)
- Concomitant medication
- Record of intercurrent or concomitant illness
- Return of the empty and unused product containers
- Patient's diary review

9.1.1 General study Schedule

Assessment	Screening visit	Randomization visit	Interim visit	EoS visit
	V0	**V1**	**V2**	**V3/FDE**
	Day 0 (-3 to -7 days)	**Day 1**	**Day 28** (± 3 days)	**Day 84** (± 3 days)
Informed Consent	X			
Inclusion/Exclusion Criteria	X	X		
Randomization		X		
Medical History	X			
Vital Signs (Tª, Blood Pressure, Heart rate)	X	X	X	X
Physical Examination	X	X	X	X
Blood Sample extraction		X	X	X
Pregnancy Test		X		
Escala de la Severidad de la Fatiga (FSS)		X	X	X
Escala Modificada de Disnea (mMRC)		X	X	X
Adverse Events		X	X	X
Concomitant medication	X	X	X	X
Concomitant diseases	X	X	X	X
Deliver of the study product		X	X	
Deliver of patient's diary		X		
Product accountability			X	X
Review of adherence to dosing schedule			X	X
Review of patient's diary			X	X
EoS = End of Study				

TABLE 1. GENERAL STUDY CHRONOGRAM.

9.2 Discussion of Study Design

This is a pilot exploratory study to test the efficacy of the IP, the food supplement, rich in omega-3 acids, on the levels of certain pro-inflammatory and pro-resolution lipid mediators and to determine the safety and tolerability of the products.

According to de exploratory nature of the study, the disease and the variables to be recorded, a 30 days' treatment is deemed sufficient to see differences between the basal values and the final values of the MRS questionnaire. If the results of the presents study point to an amelioration of the symptoms, further studies will be carried out.

The last group, the lowest dose group, group X, was not blinded due to the difficulties of masking the third active ingredient group, this group was not placebo controlled.

9.3 Selection of Study Population

The study was conducted in 53 adult patients with Post COVID-19 condition. The subjects included must have had a positive Covid-19 test (PCR, fast antigen test or serologic test) and persistent symptoms related to Covid-19 at least 12 weeks prior their enrolment in the study. The candidates were selected by the IPs directly among the patients that were treated in each centre. The IPs informed the potential candidates about the study and offered them to participate.

No study procedure was conducted before the subject had given written consent, which had to include his/her signature, name and surnames. The member of the investigation team providing the information on the study had to sign the informed consent sheet either.

The following criteria were defined to establish eligibility for study entry:

9.3.1 Inclusion Criteria

To be included in the study the participants must meet all the following inclusion criteria:

1) Adult patients with Post COVID-19 condition, both genders, between 18 and 70 years old.
    a. Patients with clinical criteria that prove the Covid-19 infection: Diagnosis confirmed using test for Covid-19: PCR, Rapid antigen test, serological test). Symptoms must persist longer than the 12 week after the beginning of the symptoms
    b. Patients with fatigue/asthenia, dyspnea and one of the following:
        i. General malaise
        ii. Headaches
        iii. Low mood
        iv. Muscular pain
2) Body mass index between 18,5 and 30 kg/m^2
3) With the ability to provide informed consent
4) Women that participate in the study must comply one of the following conditions:
    a. Unable to get pregnant: women that had surgical sterilization or over two years after menopause
    b. Fertile women must have a negative pregnancy test prior their inclusion in the study (conducted during screening) and use a highly efficient

contraceptive method, which are: hormonal contraceptives, intrauterine devices, condoms together with spermicide and gel, partner's surgical sterilization (vasectomy) or total sexual abstinence during the study. The use of these contraceptive methods must last, at least 3 months after the last dose of the study products.

9.3.2 Exclusion Criteria

To participate in the study, patients must comply with none of the following exclusion criteria:

1) Pregnant or breastfeeding women
2) Unable to use a highly efficient contraceptive method
3) Recruited in another clinical trial
4) Subjects involved in another clinical trial 4 weeks prior their inclusion
5) Patients with any concomitant illness or condition that could affect significantly the hematologic, renal, endocrine, pulmonary hepatic, gastrointestinal, cardiovascular, immunologic, central nervous, dermatologic or any other system, with the exceptions stated in the inclusion criteria
6) Use of Immunosuppressant drugs or prolonged or maintained use of anti-inflammatory drugs and/or corticoids
7) Hypersensitivity, allergy or idiosyncratic reaction to omega-3 acids. Fish or soya allergies.

9.3.3 Removal of Patients from Therapy or Assessment

Subjects were free to withdraw from the study at any time. The investigator could withdraw a subject from the study due to the onset of adverse events, or safety concerns or because of protocol non-compliance which could have jeopardized the validity of the data. Hence, a thorough monitoring, both objective and subjective of the status of each patient, their symptoms, and their adherence to the study procedures was conducted during the scheduled visits.

9.4 Treatments

9.4.1 Treatments Administered

The IP was a food supplement rich in omega-3 oil, formulated as capsules named Lipinova R-300. The detailed composition of the product is provided in Table 4. Both, the investigational product and the placebo were manufactured, packed and labelled by Laboratorios Liconsa SL.

The sponsor of the study provided all the investigational products adequately masked, except for the products for group X that was not blinded and, thus, no masked.

Group	Product	Dose	Number of capsules/8 h	
			Food supplement	Placebo
A	*"Omega-3 food supplement"* 1000 mg/8 hours	1000 mg/8h	2	0
B	*"Omega-3 food supplement"* 500 mg/8 hours	500 mg/8h	1	1
C	Placebo every 8 horas	N/A	0	2
X	*"Omega-3 food supplement"* 500 mg/24 hours	500 mg/24h	1	

TABLE 2. TREATMENT GROUPS.

The product has to be administered once o 3 times a day (depending of the assigned treatment group) for 84 days (12 weeks).

9.4.2 Identity of Investigational Product(s)

The identity of the IPs is provided in Table 3 and Table 4.

IP Name	Active Ingredient	Strength (Mg)	Pharmaceutical Form	Manufacturer	Batch Number	Expiry Date
Lipinova	Omega-3 fatty acids	503 mg	Capsule	Liconsa S.L.	20006178	06/2022
Placebo	N/A	N/A	Capsule	Liconsa S.L.	2100001	01/2023

TABLE 3 CHARACTERISTICS OF THE IP

Ingredients	mg/capsule
Active ingredients	
EPA (100-300 mg/g)	50 – 150 mg
DHA (200-450 mg/g)	100 – 225 mg
14-HDHA (40-200 mg/kg)	20 – 100 mg)
17-HDHA (80-400 mg/kg)	40 – 200 mg
18-HEPE (50-400 mg/kg)	25 – 200 mg
Total active ingredients Lipinova	503.0 mg
Ingredients of the capsule	
Bovine gelatine	150.709 mg
Glycerine (E-422)	69.291
Total	220.0
Total weight of the capsule + Active ingredients	**723.0 mg**

TABLE 4. COMPOSITION OF THE IP.

9.4.3 Method of assigning patients to treatment groups

The treatment allocation was made by randomly assigning each subject any of the treatments or the placebo group. The randomization ratio was 3:3:1:3 [16/16/5/16])

37 patients were included in groups A, B y C (randomization 3:3:1)

- Group A N = 16 patients
- Group B N = 16 patients
- Group C Placebo N = 5 patients

An additional exploratory low dose cohort was added. Due to the difficulties in masking the product this cohort is open (no masked) and not controlled.

- Group X N = 16 patients

The dosage and regime of administration of the IP for each treatment group is described in Table 2.

9.4.4 Selection of Doses in the Study

This is a pilot, exploratory study to evaluate the effect of the supplement rich in omega-3. Different daily doses were chosen. According to The EFSA the available data are not sufficient to establish a tolerable upper intake level for n-3 LCPUFA (DHA, EPA, and DPA, individually or combined) for any population group. The Panel considers that supplemental intakes of EPA and DHA combined at doses up to 5 g/day, and supplemental intakes of EPA alone up to 1.8 g/day, do not raise safety concerns for the adult population. The EFSA concluded that daily supplemental intakes of 5g of long-chain omega-3 fatty acids raise no safety concerns for adults (EFSA, 2012). The maximum daily dose of product used in this study is 3 grams of the combined oils, which is considered safe. Then, lower doses were tested to assess if there were a dose-response relationship in the effects observed.

9.4.5 Blinding

This study was double-blind and placebo-controlled. Nor the investigator or the patient knew which product was administered. Laboratorios Liconsa S.L. manufactured both, the IP and the placebo in a way that they were indistinguishable, with the same appearance and shape. The labels of all products did not reveal which contained the food supplement or which contained the placebo. The randomization list was created and maintained by member of the FTH independent of the development of the study. In order to ensure the blinding for the subjects, all subjects from groups A, B and C, had to take the same amount of capsules in each administration time: 2 capsules of food supplement (group A), 2 capsules of placebo (group C) and 1+1, food supplement and placebo (group B).

To maintain blinding to the person responsible for the bioanalysis, study samples were sent to the testing laboratory labelled with the code of the patient, the date and de code of the study.

An additional, exploratory open group (group X) was added to the study. Due to the difficulties of maintaining the blinding for this group it was decided that this would be an open, unblended group.

The blinding could have been open during the study in the following circumstances:

- Need of urgent medical treatment if it is needed to know the product administered.
- In the event of a Severe Adverse Event (SAE) that might be related to the administration of the IP should it require the expedite notification to the AEMPS.
- In the event of an Adverse Event (AE) that might require the withdrawal of a subject, according to the criteria of the investigator.

In all the above mentioned scenarios, the unbinding would only affect the concerned subject. In case of need a representative of the affected centre would have Access to the randomization code.

If it is deemed necessary to unblind some individuals to assess the evolution of the study and decide about its continuity or early termination.

9.4.6 Treatment compliance

The nutritional product was exclusively used for the present clinical trial and was only administered to the subjects enrolled in the study. The investigator was responsible for drug accountability. The investigator had to ensure that the IPs were used only in accordance with the protocol.

The subjects were instructed to return all empty and unused product containers during the interim visit and at the end of their participation. The participants were also instructed to complete a diary recording the dates and times of the administration. The amount of product dispensed and returned, including batch number and expiry date, was recorded in the CRF, and reviewed by the study monitor.

The study monitor conducted the product accountability during the monitoring visits and at the end of the study, and review the patient's diary at the end of the study. All deviations regarding the treatment compliance and drug accountability were registered in the protocol deviations table included in appendix 16.2.2.

9.4.7 Prior and Concomitant Therapy

Prior relevant medication was reviewed and recorded in the CRF before the inclusion of every subject. Only those subjects that does not take medications or products forbidden by the protocol were included in the study.

Concomitant medication was allowed in the study with the exceptions detailed in the exclusion criteria (see 9.3.2 Exclusion Criteria). All prior (relevant) and concomitant medication was documented in the CRF.

9.5 Efficacy and Safety Variables

9.5.1 Efficacy Variables

The principal efficacy variable of the study was the evolution of the pro-inflammatory and pro-resolving lipid mediator, from baseline, prior the administration of the food supplement up to the end of the study (day 84 of treatment). The following metabolites were measured both in plasma and in serum:

- Fatty acids: EPA, DHA, ARA, DPA
- Monohydroxilated SPMs: 17-HDHA, 18-HEPE, 14-HDHA
- Resolvins: RvE1, RvD1, RvD2, RvD3, RvD4, RvD5
- Maresins: MaR1, MaR2
- Protectins: PD1, PDX
- Lipoxins: LXA4, LXB4
- Prostaglandins: PGE2, PGD2, PGF2α
- Thromboxanes: TXB2
- Leukotrienes: LTB4

As secondary efficacy objective, the evolution of the above mentioned parameters until the fourth week of treatment (day 28) was calculated.

Other secondary efficacy variables are:

- Fatigue Severity Scale (FSS) test: The FSS test measures fatigue in a unidimensional scale. It consists in 9 questions with 7 possible answers each which quantifies each item in a 1 to 7 scale. The evolution of the mean scores from baseline to visit 2 (4^{th} week of treatment, day 28) and to the end of the study (day 84 of treatment) is calculated.
- Modified Medical Research Council (mMRC) Dyspnea Scale: The scale includes 5 degrees of physical activity that could cause dyspnea. The scale punctuates the dyspnea in a range from 0 (No exercise cause dyspnea) to 4 (the dyspnea prevents the patients going out of the house or performing routine daily activities like dressing up. The baseline results are compared to the scores at visit 2 (day 28) and at the end of the study (day 84).

9.5.2 Safety Variables

To assess the safety of the IP, all Adverse Events (AEs) that occur to the participants during the study, since the first administration of the IP up to the last visit (treatment emergent AEs), were collected, assessed and recorded in the CRF, regardless of their relationship to

the study product. The events that would have begun before the star of the treatment were included as part of the clinical history of the subject.

The AEs could be clinically significant abnormalities found in the vital signs (body temperature, heart rate or blood pressure) or during the physical exam, or could be reported directly by the subjects to the investigators either during the visits or through their diaries. The investigators had to record the AEs in the CRF, assess their intensity, seriousness and casual relationship with the IP using their best medical judgement and experience.

The AEs are coded according to MedDRA Version 25.1.

9.5.2.1 AE definition

For the purposes of the study an AE is defined as any untoward medical occurrence in a subject to whom the IP was administered and which did not necessarily have a causal relationship with this product. This means that any occurrence starting prior the administration of the IP was not considered an AE, but as part of the medical history of the subject.

9.5.2.2 Reporting Period

For reporting purposes, only treatment emergent AEs are considered. Those events were recorded as such since the first administration of the food supplement up until the last visit, at day 84. Events that start after the last day of the study would only be reported if the PI states a clear causal relationship between the IP and the event.

9.5.2.3 Causality

The potential causal relationship of the IP to an AE was rated according to the following 3-point scale:

- Not related: The temporal sequence makes extreme unlikely that the event and the administration of the IP could be related. There are other more plausible causes like the administration of other drugs, other therapeutic interventions or underlying circumstances that are more likely to cause the event.
- Related: There is a clear temporal sequence that links the administration of the IP and the onset of the clinical event. Other concomitant medication, therapeutic interventions or underlying circumstances do not provide a sound explanation for the event.
- Suspected: There is a reasonable probability that the event was caused by the administration of the IP. «Suspected» implies that there are no certainties and some doubts exists about the cause of the event.

For the purposes of the study, the related or suspected events are considered Adverse Reactions (ARs)

9.5.2.4 SAEs

A Serious Adverse Event is defined as any untoward medical occurrence that at any dose:

- results in death (considering death an effect not an occurrence),
- Is life-threatening. Life-threatening refers to an AE in which the subject was at immediate risk of death at the time of the event. It does not refer to an event, which may have caused death, if it was more severe.
- requires inpatient hospitalization or prolongation of existing hospitalization: Refers to a hospitalization that has not been scheduled before the entry of the subject in the study, and that is prolonged overnight. Hospital admissions and/or surgical operations planned before study inclusion are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that the condition did not deteriorate during the study.
- results in persistent or significant disability/incapacity: Significant alteration of the patient's ability to perform routine daily activities.
- is a congenital anomaly/birth defect: affecting the offspring of a subject that has taken the IP, regardless of the time of the diagnosis.
- It is an important medical event: Medical and scientific judgement was used to decide if an expedite notification was required for a particular AE. Important medical events are those that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above.

9.5.2.5 Information to be Provided by the Investigator for a SAE

The FTH provided with appropriate forms to record the information related to the SAEs. The information to be provided include the identification of the subject, onset date and end, relevant test done and their results, concomitant medication used to treat the event, assessment of causal relationship with the IP and outcome.

9.5.3 Appropriateness of Measurements

The Fatigue Severity Scale (FSS) is a well-known and widely used method of evaluating the fatigue as a symptom. Although the scale was initially validated in a population of patients with multiple sclerosis and systemic lupus erythematosus it is now used on a variety of different chronic conditions and disorders. The FSS is a short questionnaire that requires the subject to rate his/her level of fatigue using nine statements that rate the severity of the fatigue symptoms. Although its main weakness is its subjectivity, there are some advantages, as it is brief and easy to do, it measures not just fatigue but the effect of fatigue on function, and has been widely used both clinically and in research.

The modified Medical Research Council (mMRC) scale is a five-level rating scale based on the patient's perception of dyspnea in daily activities. It is a simple and valid tool to assess disability and it is the most commonly used validated scale to assess dyspnea in daily living in chronic respiratory diseases.

Both combined are considered an appropriate, tested and reliable way to measure the relief of some of the most common Post COVID-19 symptoms, dyspnea (43% of the patients) and fatigue (53% of the patients), and measure the clinical effect of the food supplement over the length of the study.

The omega-3 fatty acid have shown the ability to increase SPMs levels both in serum and plasma which in turn could affect the inflammatory state of the patient and the clinical evolution of patients with Post COVID-19 condition. The measurement of the plasma and serum concentrations of pro-inflammatory (prostaglandins and Leukotrienes) and pro-resolving lipid mediators (lipoxins, resolvins, protectins, maresins and Monohydroxilated mediators derived from EPA and DHA) in patients with Post COVID-19 condition, provided a very valuable information about the immunological response of the patients regarding the inflammatory condition caused by the viral infection.

9.5.4 Drug Concentration Measurements

Does not apply

9.6 Data Quality Assurance

Appropriate actions to guarantee the quality of the data register were applied. This guarantees that data were collected and processed in a truthful and correct way. Once finalized the clinical phase of the study and after revision by the CRA, original CRFs have been sent to the sponsor.

Monitoring was performed by Fundación Teófilo Hernando (FTH). 100 % Source Data Verification was done.

9.7 Statistical Methods Planned in The Protocol and Determination of Sample Size

9.7.1 Sample Size

This is an early proof-of-concept study. It has been designed as a pilot study whose principal objective is to determine the effect of the food supplement on some lipid mediators that are involved in inflammatory process, thus, no formal sample size calculations have been done.

9.7.2 Analysis Sets

The following study populations are defined for the analysis of the results:

- Intention To Treat population (ITT): includes all randomized patients. The analysis is done according to the initial group to which the patient was assigned.
- Per Protocol population (PP): includes those patients that have ended the study following all the procedures and visits stated in the protocol with no mayor protocol violations.

To assess the differences between these two groups, cases of loss of follow-up and the causes (if possible) are recorded and reported for each treatment group.

The safety analysis includes all subjects that have received at least one dose of the food supplement or the placebo (Safety Set [SS]).

9.7.3 Patient Demographics/other Baseline Characteristics

Assessments performed prior to randomization, at Screening and Baseline were displayed using summary statistics at the conclusion of this study. These include: Age, race, prior relevant medical events, family record, weight, and Physical Examination. Treatment groups and subject populations were identified. No formal statistical analyses were planned.

Baseline data, regarding the objectives of the study were also recorded:

- Serum and plasma concentrations of the pro-inflammatory and pro-resolving lipid mediators
- Fatigue Severity Scale (FSS) test
- Modified Medical Research Council (mMRC) Dyspnea Scale

9.8 Changes in The Conduct of the Study or Planned Analyses

In order to obtain a better understanding of the physiological effects of the food supplement on the serum and plasma concentrations of the lipid mediators, and the inflammatory status of the patients, the variables are grouped into different categories and the ratios of pro-inflammatory and anti-inflammatory ratios were analysed and discussed:

- Pro-resolving/anti-inflammatory variables:
    - Fatty acids: EPA, DHA, ARA, DPA and
    - Monohydroxilated SPMs: 17-HDHA, 18-HEPE, 14-HDHA
    - Resolvins: RvE1, RvD1, RvD2, RvD3, RvD4, RvD5
    - Maresins: MaR1, MaR2
    - Protectins: PD1, PDX
    - Lipoxins: LXA4, LXB4
- Monohydroxilated SPMs: 17-HDHA, 18-HEPE, 14-HDHA
- 14-HDHA Metabolome: MaR1+MaR2
- 17-HDHA metabolome: RvD1 + RvD2 + RvD3 + RvD4 + RvD5

- Pro-inflammatory variables
    - Prostaglandins: PGE2, PGD2, PGF2α
    - Thromboxanes: TXB2
    - Leukotrienes: LTB4
- Prostaglandins = PGE2 + PGD2 + PGF2α

Also the following ratios were calculated and compared between groups and/or between baseline and visit 2 and 3

- Pro-resolving/anti-inflamatory:Pro-inflammatory variables
- Monohydroxilated SPMs:Por-inflammatory variables
- 14-HDHA Metabolome:Prostaglandins
- 17-HDHA Metabolome:Prostaglandins

There were also changes in the number of patients per group. According to the randomization scheme described in the protocol groups A, B and X should have included 16 subjects whereas group C should have included 5 subject. Subject 0303 was randomized to the placebo group but, due to an error in the study centre, it was included in group A, and 1500 mg/day of IP was administered. Therefore, groups A and X had the planned number of subjects, 16, whereas, group B has 16 (one more), and group C has one subject less (N=4).

10 STUDY PATIENTS

A total of 54 patients were informed and screened for the study, only one, screening ID Z2-05, was excluded before randomization due to a screening failure. The patient did not meet inclusion criteria No 2, BMI > 30 kg/m^2.

53 patients were randomized and received at least one dose of the food supplement, 48 were included in centre No 2 and 5 in centre No 3. Centre No 1 did not include any subject nor conducted any screening procedures.

3 of the subjects abandoned the study before its completion, all due to AA:

- Subject 204 due to a SAE reported on the 04/12/2021
- Subject 212 due to a ligament sprain on the 05/03/2022 that impeded the subject to attend to the study visits
- Subject 234 was withdrawn due to polymenorrhoea and heavy menstrual bleeding considered possibly related to the IP.

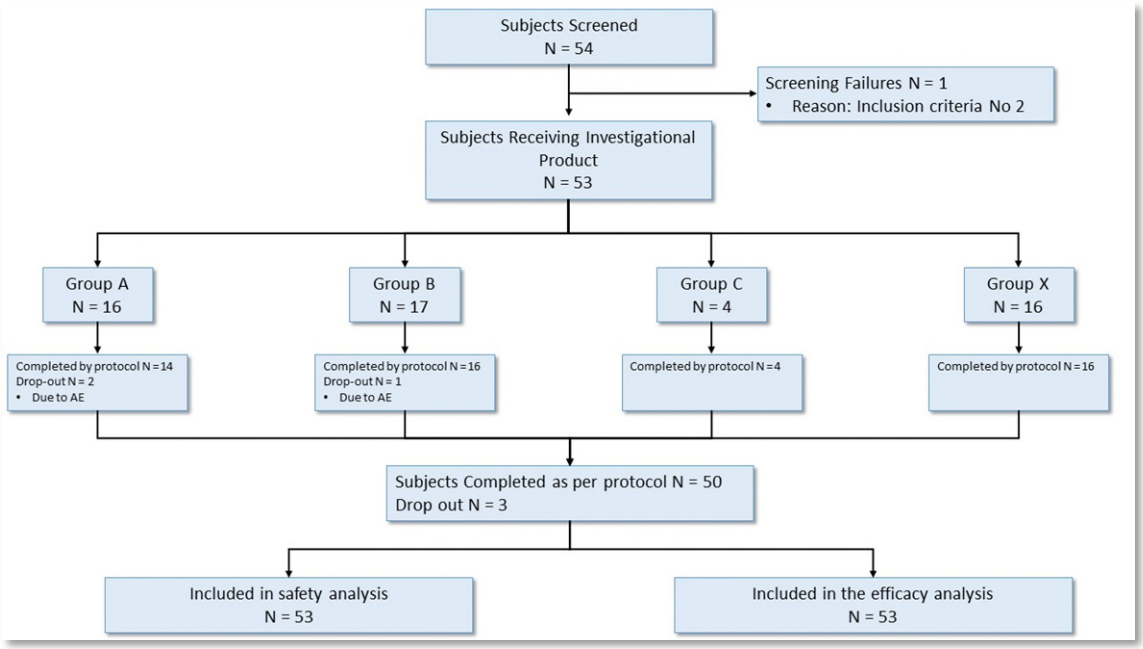

FIGURE 1 DISPOSITION OF SUBJECTS

10.1 Protocol Deviations

A total of 80 protocol deviations were detected during the study, 62 occurred in centre 2 and 18 in centre 3. Most of them were considered minor, as they did not affect the validity of the data or the rights and wellbeing of the participants. Only 1 deviation was considered major, patient 2-31 forgot to declare that he/she took inhaled corticosteroids occasionally before and during the study, which are prohibited medicaments according to the inclusion/exclusion criteria (Criterion No 6: Use of Immunosuppressant drugs or prolonged or maintained use of anti-inflammatory drugs and/or corticoids). In any case, after consultation with the sponsor it is not likely that the use of such a drug, in the regime declared by the subject would have a significant effect on the patient results, thus the results are included in the efficacy analysis.

Centre	ID	Date	Visit	Deviation	Type	Remark
2	2-31	-	-	Patient occasionally took inhaled corticosteroids, thus meeting exclusion criterion 6.	Major	During visit 3, patient explained that began taking inhaled corticosteroids before his/her inclusion in the study, but forgot to mention it.

TABLE 5 MAJOR PROTOCOL DEVIATIONS

There were no critical deviations to the protocol or the Spanish legislation during the study.

A complete listing of protocol deviations is provided in Appendix 16.2.2.

11 EFFICACY EVALUATION

11.1 Data Sets Analysed

2 study populations were defined as follows:

- Safety population: All patients receiving any investigational product were included in the safety analysis
- Efficacy population: All patients that fulfil all study inclusion/exclusion criteria and have, at least one post-treatment efficacy observation, were included in the efficacy analysis. All available data obtained from the subjects that abandoned the study before the was used.

The data from the patient that was supposed to receive placebo (group C) and was mistakenly included in group B (and therefore receive the medium dose), was analysed according to the group he/she was included and the treatment received.

11.2 Demographic and Other Baseline Characteristics

The demographic characteristics of the participants, including their most relevant medical history and baseline vital signs are detailed in appendix 16.2.4.

Only patients with Post COVID-19 condition (infection confirmed and symptoms lasted at least 12 weeks before their inclusion) were included in the study. The average age of the patients was 50.1 years, with a minimum of 32 and a maximum of 70 years.

11.3 Measurements of Treatment Compliance

The study subjects were instructed to return all the investigational product left, including the empty bottles. The site keep records of the product sent by the sponsor and returned. The amount of investigational product given to the subjects, and returned to the investigators, including the batch and the expiration date, was recorded in the CRF.

All patients received a diary at the beginning of their participation in the study, together with the food supplement supplies. The patients should have recorded the daily administration of the IP and any possible deviation in the administration. The PI reviewed each patient`s diary during the subjects scheduled visits.

The Sponsor’s designated Monitor verified the product accountability during periodic monitoring visits and the patients’ diaries at the end of their participation in the study. The deviations regarding the compliance with the treatment and accountability of the product are detailed in the deviation list (appendix 16.2.2 Protocol deviations).

11.4 Efficacy Results and Tabulations of Individual Patient Data

11.4.1 Primary objective – Evolution of pro-inflammatory and pro-resolution mediators.

The primary objective of the study is to compare the evolution of serum and plasma concentration of pro-inflammatory and pro-resolving lipid mediators in patients with Post COVID-19 condition after the daily administration of a food supplement of a food supplement, rich in omega-3 fatty acids. The analysis was done 12 weeks after the beginning of the treatment (principal objective) and 4 weeks after the first dose (secondary objective)

The determinations of the plasma and serum concentrations were done by Solutex following their own protocols.

The blood samples were extracted at baseline, and 4 weeks (for the secondary objective) and 12 weeks of treatment.

The pro-inflammatory and pro-resolving lipid mediators that were considered were:

- Fatty acids: EPA, DHA, ARA, DPA
- Monohydroxilated SPMs: 17-HDHA, 18-HEPE, 14-HDHA
- Resolvins: RvE1, RvD1, RvD2, RvD3, RvD4, RvD5
- Maresins: MaR1, MaR2
- Protectins: PD1, PDX
- Lipoxins: LXA4, LXB4
- Prostaglandins: PGE2, PGD2, PGF2α
- Thromboxanes: TXB2
- Leukotrienes: LTB4

Homogeneity analyses were done with the data recorded at the time of randomization to ensure that can be compared among groups. Differences between groups were calculated using an ANOVA test using correction for multiple comparisons. Changes on the principal efficacy variables were assesses and the data from the 4 treatment groups were compared using a Generalized linear model.

Mean differences between baseline and weeks 4 and 12 were calculated for each one of the pro-inflammatory and pro-resolving lipid mediators

Composite variables were also included in the analysis. The variables were divided in two groups, those who are responsible of the maintenance of the chronic inflammatory response and those that helps to cease it, whose data were normalized and then added:

- Pro-inflammatory mediators: PGE2, PGD2, PGF2α, TXB2 and LTB4
- Pro-resolving / anti-inflammatory mediators: EPA, DHA, ARA, DPA, 17-HDHA, 18-HEPE, 14-HDHA, RvE1, RvD1, RvD2, RvD3, RvD4, RvD5, MaR1, MaR2, PD1, PDX, LXA4, LXB4

For the statistical report, the values used to calculate the composite variables were normalized subtracting to each individual value the mean value for the variable and dividing the result by the standard deviation, so all variables can be added in a normalized common scale.

The ratio between pro-inflammatory and pro-resolving mediators was also calculated.

All patients with the analytical analysis done at baseline and visit 3 (week 12 of the study) have been included in the statistical analysis. Two different analysis were done one using the concentration of the metabolites in serum and other with the concentration of the metabolites in plasma.

11.4.1.1 Serum

Regarding the primary endpoint of the study, the evolution of pro-inflammatory and pro-resolution mediators after 12 weeks of treatment, there were no differences in the majority of the metabolites studied. Group A is the only where all pro-inflammatory means are reduced. At 12 weeks all groups, even the placebo (C) group experience a reduction on the pro-inflammatory and an increase in the pro-resolving. But only the ratio Anti:Pro is positive for groups A and C, showing no apparent trends nor dose response relationship.

Overall all PUFAs and the monohidroxilated mediators 14-HDHA, 17-HDHA and 18-HEPE, tend to increase during the study in all groups. The highest dose group experienced a highest, statistically significant increase of 18-HEPE at 12 weeks compared with the rest of the groups.

The intragroup differences are statistically significant for 17-HDHA between baseline and the second and third visit in the highest dose group and between baseline and the last visit in the medium dose group (Figure 2). Likewise, 18-HEPE increases significantly between baseline and the 2nd and last visit in the two highest dose group but not in the lowest dose group (Figure 2). There were no significant differences after the IP consumption for 14-HDHA in none of the groups.

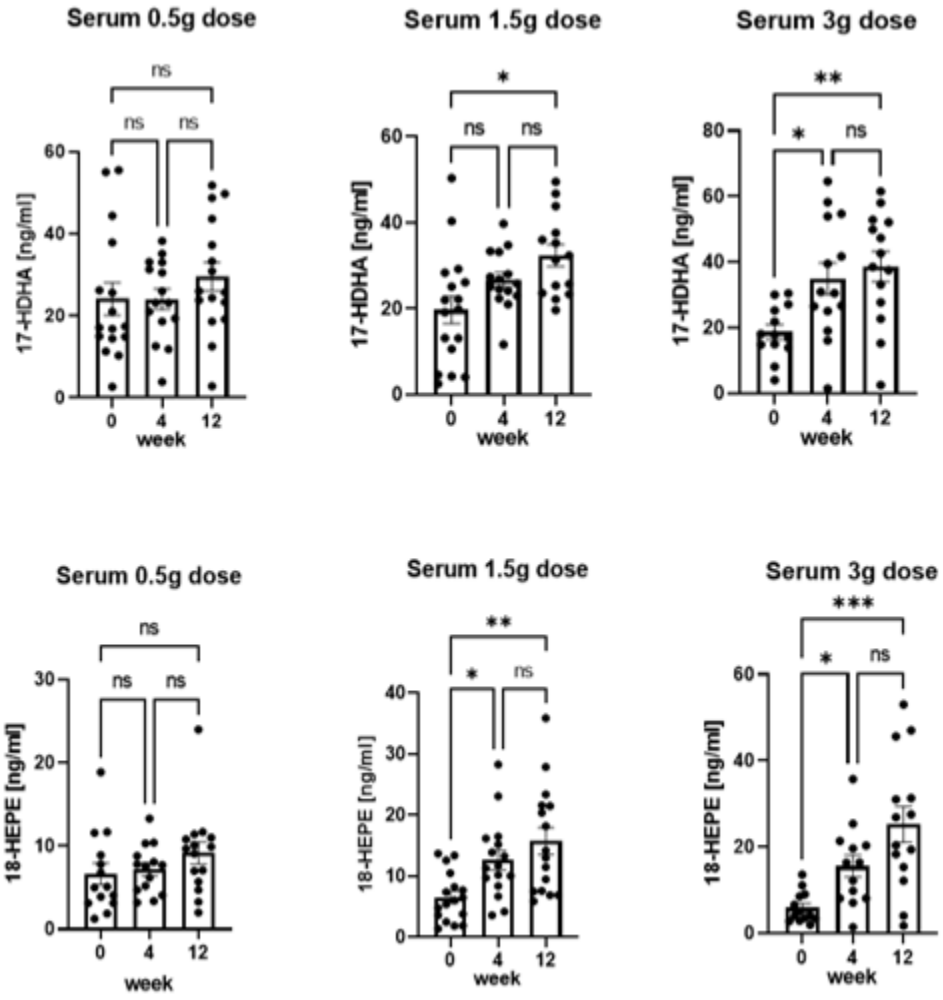

FIGURE 2. EVOLUTION OF 17-HDHA AND 18-HEPE CONCENTRATIONS IN SERUM. *P<0.05.

When analysed individually, the SPMs maresins, resolvins, protectins and Lipoxins, tend to experience slight non-significant changes, mostly increases with no apparent differences among the treatment groups.

In relation with the composite variables, at 4 weeks, anti-inflammatory mediators rise in groups A, B and C and decrease in the placebo group. At week 12 anti-inflammatory variables rise and the pro-inflammatory decrease in all groups. When comparing the evolution of the composite variables between the treatment groups at the end of the study, the pro-inflammatory mediators were significantly lower in the 3g/day group compared with groups C (Placebo) (p=0.036) and B (p=0.04), but not with group X (the lowest dose group) (Table 12 and Table 13).

Ratio monohydroxylated SPM precursors Vs pro-inflammatory mediators in serum.

To compare the evolution of the inflammatory status of the patients and their capability of resolution, the ratio of SMPs and pro-inflammatory mediators was compared for the 3 active ingredient groups over the course of the study. The total concentration of each pro-resolution monohidroxilated SPM, 14-HDHA, 18-HEPE and 17-HDHA was added and divided by the total number of pro-inflammatory metabolites, PGE2, PGD2, PGF2α, TXB2

and LTB4 (Table 6). In this case the mean of the variables were added directly and not normalized prior the sum.

Serum	Group X (500mg)			Group B (1500mg)			Group A (3000mg)		
	Baseline	Visit	%	Baseline	Visit	%	Baseline	Visit	%
Baseline-Visit 2 (week 4)									
Monohidroxilated	167.3	201.9	21%	169.9	225.4	33%	184.4	250.1	36%
Monohidroxilated/pro-inflammatory	2.20	2.28	4%	2.65	2.82	7%	1.14	1.57	38%
Visit 2 (week 4) - Visit 3 (week 12)									
Monohidroxilated	201.9	214.8	6%	225.4	278.1	23%	250.1	290.9	16%
Monohidroxilated/pro-inflammatory	2.28	3.19	40%	2.82	4.14	47%	1.57	5.62	259%
Baseline - Visit 3 (week 12)									
Monohidroxilated	167.3	214.8	28%	169.9	278.1	64%	184.4	290.9	58%
Pro-inflammatory/monohidroxilated	2.20	3.19	45%	2.65	4.14	56%	1.14	5.62	395%

TABLE 6. EVOLUTION OF MONOHYDROXYLATED SPM PRECURSORS AND PRO-INFLAMMATORY MEDIATORS RATIO

The total serum SPMs Precursors:pro-inflamatory ratio increases during the study in a dose dependent manner for all doses used (Figure 3). The more significant increment of the ratio occurs in the late stages of the study, between visits 2 (after 4 weeks of treatment) and 3 (12 weeks after the beginning of the treatment). The ratio pro-resolution:pro-inflammatory increases, during the last period, a 259% for the highest dose group (A), 47% for the medium dose group (B), and 40% for the lowest dose group (X).

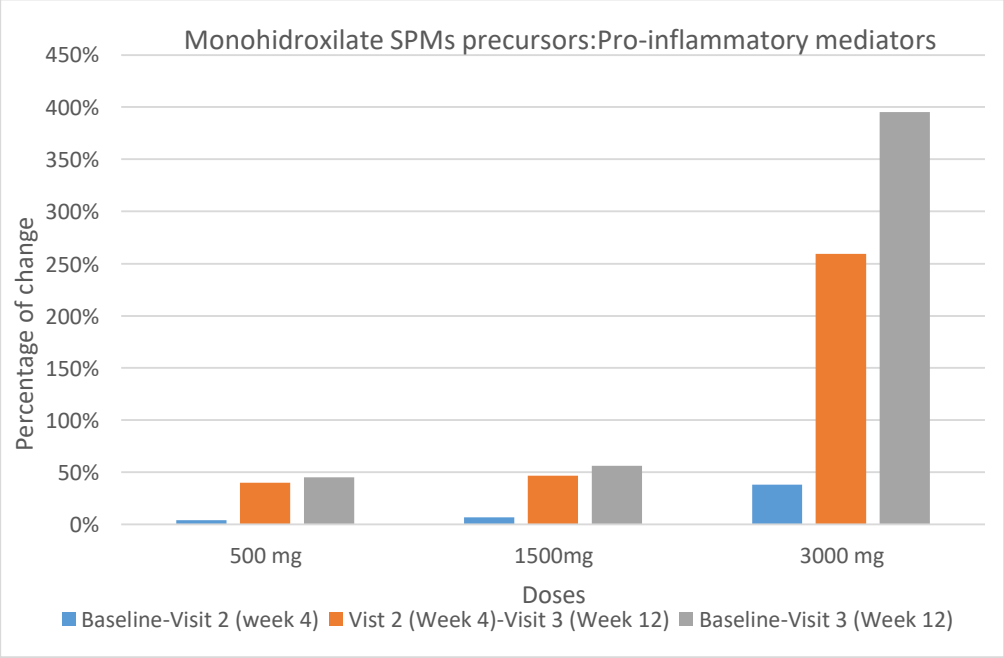

FIGURE 3. PERCENTAGE OF CHANGE: MONOHIDROXYLATED SPMS PRECURSORS VS PRO-INFLAMMATORY MEDIATORS.

Composite metabolomes vs prostaglandins

Being the prostaglandins one the most relevant determinants for the evolution of the inflammatory response, the ratio of their concentrations in serum and plasma was compared with the 14-HDHA, 17-HDHA and 18-HEPE metabolomes during the study.

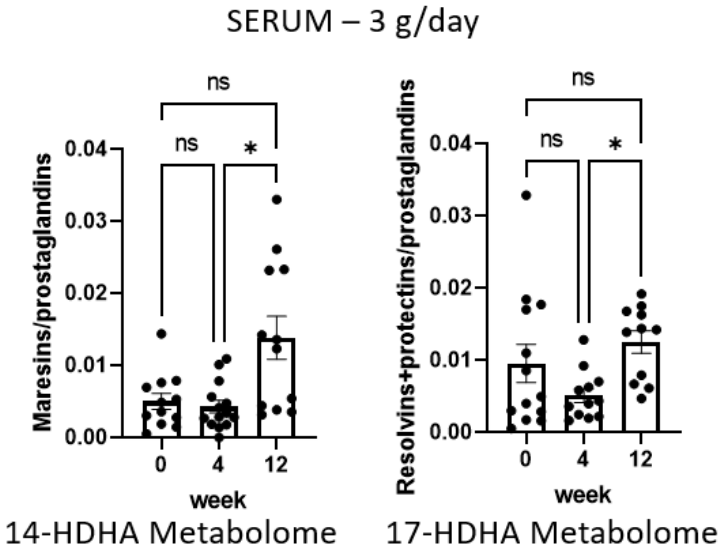

FIGURE 4. RATIO 14-HDHA AND 17-HDHA METABOLOMES:PROSTAGLANDINS IN SERUM FOR THE 3G/DAY GROUP. * P<0.05.

14-HDHA metabolome comprises Maresins 1 and 2, 17-HDHA metabolome comprises resolvins Resolvins, RvD1, RvD2, RvD3, RvD4, RvD5 and protectins PD1 and PDx, whereas 18-HEPE metabolome comprises RvE1. There were no significant differences between baseline and visit 2, where a slight decrease in the ratio is observed, or baseline and visit 3 in none of the ratios, however, statistically significant differences were found between weeks 4 and 12 (Figure 4), which points to a delayed action of the IP, which exerts its action after several weeks of treatment. During the first weeks of the study the No significant differences were observed regarding the 18-HEPE metabolome.

11.4.1.2 Plasma

Regarding the primary endpoint of the study, the evolution of pro-inflammatory and pro-resolution mediators after 12 weeks of treatment, there were no differences in the majority of the metabolites studied. The mean of the Fatty acids, EPA, DHA, ARA, DPA, increased slowly in all treatment groups. Only in the highest dose group the mean differences indicate higher values in the first visit. In the placebo and the 0.5 mg/day groups all values descend during the first (4 weeks), and all but DPA do the same in the 1.5g/day group.

The levels of 18-HEPE, 17-HDHA and Mar1 experimented a significant increase in group A, at week 12, when compared with the lowest dose groups, X and B, but not to group C (placebo), where online the increase in Mar 1 was statistically significant. Moreover, there was a significant reduction of the pro-inflammatory mediators, PGE2 y PGF2a between

groups A (3g/day) and X (1.5g/day) and the placebo. There were no relevant differences between groups at week 4.

For the rest of the analyzed metabolites, it does not appear to be a clear individual pattern of change that identify with the different dosing groups, nor a dose response activity of the IP over their concentrations

When looking at the monohidroxilated SPMs concentrations, the intragroup differences were statistically significant for 17-HDHA between baseline and week 12, for the highest doses, but not for the lowest dose (Figure 5). 18-HEPE increases significantly between baseline and the las visit for all doses and between the baseline and week 4 for the two highest doses groups. There were no differences between week 4 and week 12 (Figure 5). No differences were observed in the 14-HDHA concentrations in neither of the groups.

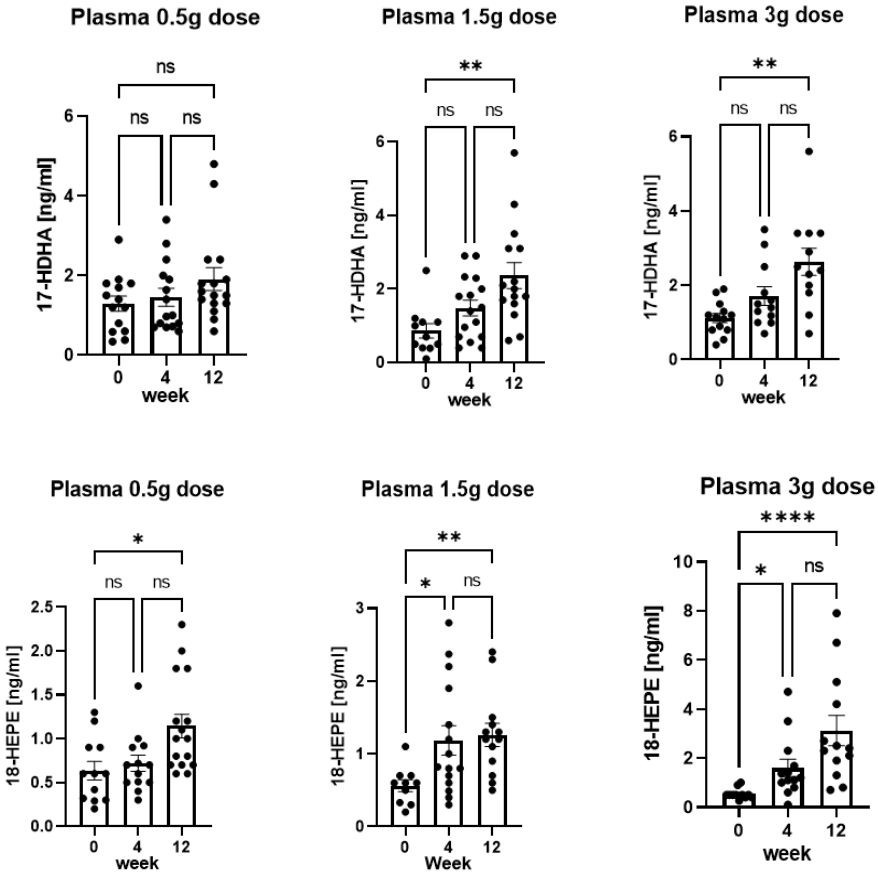

FIGURE 5. EVOLUTION OF 17-HDHA AND 18-HEPE CONCENTRATIONS IN PLASMA.

Regarding the composite variables, at 4 weeks, anti-inflammatory and pro-inflammatory mediators rise all groups but the placebo group. The ratio increases in groups A, B and C and decreases in group X. At week 12 anti-inflammatory variables rise in all IP groups and decrease in the placebo group and the pro-inflammatory increase in all groups. The ratio Pro:Anti only rise in the highest dose group. When comparing the evolution of the composite variables between the treatment groups at the end of the study, the pro-inflammatory mediators were significantly lower in the 3g/day group compared with groups C (Placebo) (p=0.036) and B (p=0.04), but not with group X (the lowest dose group) (Table 11 and Table 12).

Composite metabolomes vs prostaglandins

The evolution of ratio of the composite the 14-HDHA, 17-HDHA and 18-HEPE metabolomes Vs prostaglandins was compared with during the study.

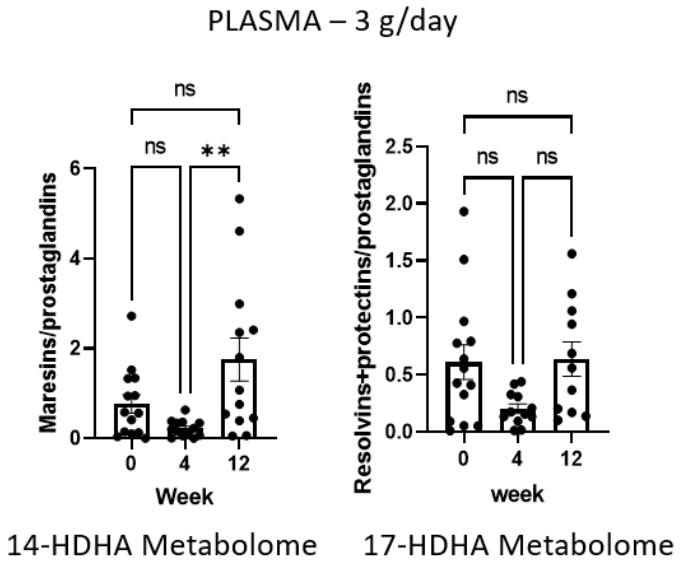

FIGURE 6. RATIO 14-HDHAA AND 17-HDHA METABOLOMES:PROSTAGLANDINS IN PLASMA FOR THE 3G/DAY GROUP. * P<0.05

There were no significant differences between baseline and visit 2, where a slight decrease in the ratio is observed, or baseline and visit 3 in none of the ratios for all 3 metabolomes. Statistically significant differences were found between week 4 and 12, but only for the 14-HDHA metabolome (Figure 6), not for the 17-HDHA or the 18-HEPE metabolome.

11.4.2 Secondary efficacy objectives

Long COVID-19 or persistent COVID-19 has a large variety of clinical manifestations. Patients that suffer from this condition experience multisystemic symptoms that have a great impact in their quality of life. A survey conducted by the Spanish society of general practitioners and family doctors (SEMG) among 1834 subjects, identified up to 201 different symptoms with a mean length of 6.2 months. 50% of the subjects that participated in the survey reported up to 58 different symptoms with a mean of 36. The five most common symptoms described by the questioned patients were asthenia/fatigue (95,9%), headache (86,5%), low mood (86,2%), myalgia (82,8%), dyspnea (79,3%) (Pilar RL et al. 2021).

The hypothesis of the study is that the food supplement could have a positive effect over the chronic inflammatory process, which is maintained in long COVID-19 patients, through the variation of the ratio of pro-resolution and pro-inflammatory mediators, and which in turn should have a clinical meaning and impact patient's symptoms and quality of life. To determine the effect of the IP on the clinic manifestation of long COVID-19, as secondary

objectives of the study, its effects on the patient's fatigue and dyspnea, two of the most prevalent symptoms observed in these patients, was assessed. The secondary efficacy variables are:

- Changes in the Fatigue Severity Scale (FSS) scores from baseline until weeks 4 and 12
- Changes form baseline until weeks 4 and 12 in the mMRC (Modified Medical Research Council) Dyspnoea Scale.

Both scales are commonly used and validated methods to asses either fatigue or the degree of functional disability due to dyspnoea.

The evolution of these clinical variables was analyzed including the 4 groups of treatment using a mixed general linear model.

Differences between the baseline FSS scores and 4 and 12 weeks after treatment were calculated. All groups show a tendency to improve the fatigue symptoms included in the FSS questionnaire (Figure 7), but no significant differences are detected among the 4 treatment groups.

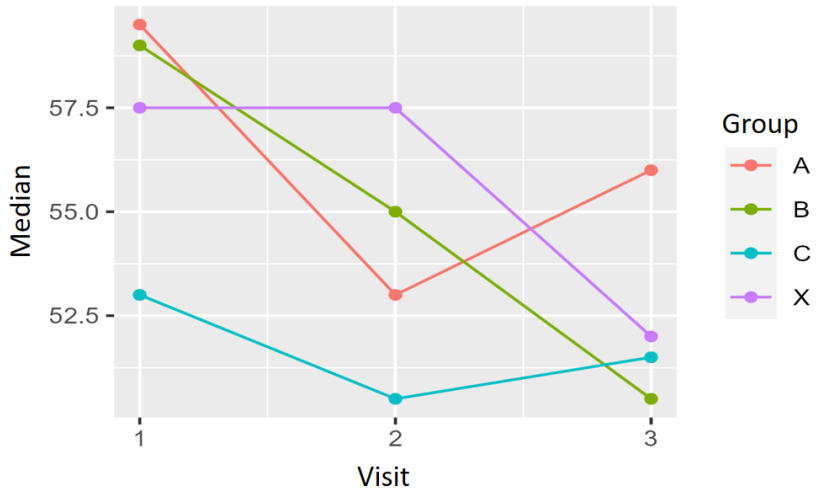

FIGURE 7. EVOLUTION OF THE MEDIAN VALUES OF THE FSS SCALE.

Differences between baseline and week 4 and 12 in the mMRC scale scores were calculated for each individual patient. The Chi-square was used for the analysis of the differences between treatments. For the differences between baseline and week 12, X-squared = 8.2496 and p-value = 0.509, and between baseline and week 4 X-squared = 7.3615 and p-value = 0.600. A slight improvement can be observed for each group, in terms of frequency and percentage of patients in each grade of the scale, but there were no significant differences in the evolution of the mMRC scores among the 4 treatment groups during the study.

Figure 8 shows the evolution of the mMRC scores for each treatment group at baseline (1), after 4 weeks of treatment (2) and at the end of the study, after 12 weeks of treatment (3). Data revealed an overall slight improvement in the MMRC scale in all groups at the end of the study (Table 7), most of the patients included experienced none or 1 point of improvement. Analysis revealed no differences among the study groups.

Changes between week 12 and Baseline **Number and % of patients that have experienced changes in mMRC score: -2, -1, 0 or 1**					
Treatment	**-2**	**-1**	**0**	**1**	**Total**
A	0 (0)	5 (33.33)	10 (66.67)	0 (0)	15 (100)
B	2 (12.5)	6 (37.5)	7 (43.75)	1 (6.25)	16 (100)
C	0 (0)	2 (50)	2 (50)	0 (0)	4 (100)
X	3 (18.75)	8 (50)	5 (31.25)	0 (0)	16 (100)
Data are displayed as N (%). Chi-square: X-squared = 8.2496, df = 9, p-value = 0.509					

TABLE 7. CHANGES IN THE MMRC SCORE BETWEEN BASELINE AND VISIT 3.

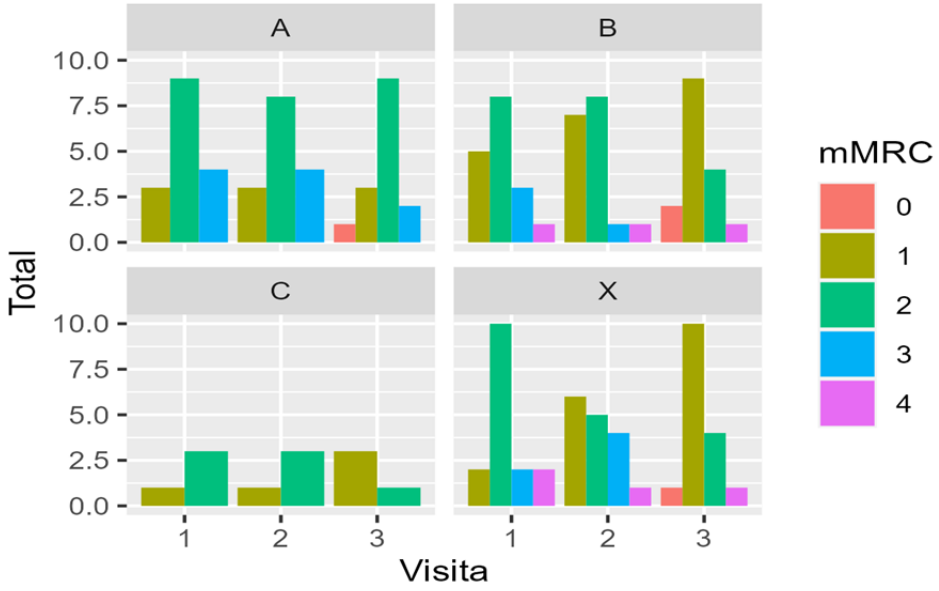

FIGURE 8. MMRC SCORES DISTRIBUTION FOR EACH VALUE IN THE 4 TREATMENT GROUPS.

A thorough analysis of the mMRC and FSS data can be found in appendix 16.2.6

12 SAFETY EVALUATION

The safety evaluation of the product was done through the analysis of the Adverse Events (AEs) that were registered during the study. The number, frequency, seriousness and their relationship with the investigational product is evaluated. All AEs reported by the subjects included were registered in the CRF. The study subjects were instructed to record in their diaries all events that might affect their health; also, during each visit, all participants were asked about any adverse event that they may have experienced. Adverse events spontaneously reported by the volunteers were also registered. Data from all patients that receive at least one dose of the study products were included in the safety evaluation.

Only the treatment emergent adverse events, those whose onset is dated after the administration of the first dose of the study product, are taken into consideration for the safety analysis of the IP.

12.1 Extent of Exposure

53 patients were included in the study and received at least one dose of the IP. 50 patients complete the study as per protocol and continue with the treatment for 12 weeks. All deviations regarding the product administration, detected during the product accountability procedures were detailed in the protocol deviations table included in appendix 16.2.2.

- 16 patients were included in group A and took 3000 mg of IP a day (1000 mg / 8 hours) during 12 weeks. 14 of them completed the study as per protocol.
- 17 patients were included in group B and took 1500 mg of IP a day (500 mg / 8 hours) during 12 weeks. 16 of them completed the study as per protocol.
- 16 patients were included in group X and took 500 mg of IP a day during 12 weeks. All of them completed the study as per protocol

3 patients abandoned the study before its completion due to the onset of AA, 2 that were assigned to group A and one that was assigned to group B.

- Subject 02-04: Abandoned the study 10 days after the start due to an AA
- Subject 02-12: Abandoned the study due to an AE and did not attend to visit 3
- Subject 02-34: Abandoned the study due to an AE after 68 days of treatment.

12.2 Adverse Events (AEs)

12.2.1 Brief Summary of Adverse Events (AEs)

A summary of adverse events is provided in Appendix 16.2.7 Adverse Events Listing (each subject). This appendix includes all AEs registered during the study sorted by treatment group. and coded according to MedDRA version 25.1.

Out of the 53 subjects included 31 (58.5% of the total), reported at least one AE. A total of 108 AEs were registered (Table 8). The intensity of most of the AEs, 96 events (88.9%), were assessed as mild, 9 (8.3%) were considered moderate and 3 (2.8%) severe, 2 caused by a traffic accident, that caused the hospitalization of the subject: road traffic accident and lower limb fracture, and one arthralgia; all considered unrelated to the IP. There were no life-threatening events.

91.7% of the events (N=99) were considered unrelated to the IP and in 8.3% possibly related, all assessed as mild. All related events were reported by subjects included in the highest dose groups, group A (3000 mg/day), 5 evens reported by 3 subjects, and B (1500 mg/day), 4 events reported by 2 subjects. No related events were reported in the placebo group (group C), nor in the lowest dose group (group X – 500 mg/day) (Table 9).

	Treatment group				Total (N=53)
	A 1000 mg/8h (N=16)	B 500 mg/8h (N=17)	C Placebo (N=4)	X 500 mg/24h (N=16)	
Number of subjects with at least 1 AE (percentage)	7 (43.8%)	10 (58.8%)	3 (75.0%)	11 (68.8%)	31 (58.5%)
Total number of AEs	34	38	10	26	108
Mild	30	36	9	21	96
Moderate	2	2	1	4	9
Severe	2	0	0	1	3

TABLE 8. OVERALL INCIDENCE OF ADVERSE EVENTS REGARDLESS OF THEIR RELATIONSHIP TO THE STUDY TREATMENT.

There were no life threatening Adverse Events during the study.

	Treatment group				Total (N=53)
	A 1000 mg/8h (N=16)	B 500 mg/8h (N=17)	C Placebo (N=4)	X 500 mg/24h (N=16)	
Number of subjects with at least 1 AE (percentage)	3 (18.8%)	2 (11.8%)	0 (0%)	0 (0%)	5 (9.4%)
Total number of AEs	5	4	0	0	9
Mild	5	4	0	0	9
Moderate	0	0	0	0	0
Severe	0	0	0	0	0

TABLE 9. OVERALL INCIDENCE OF ADVERSE EVENTS CONSIDERED POSSIBLY OR PROBABLY RELATED TO THE STUDY MEDICATION.

12.2.2 Display of Adverse Events

A complete list of adverse events, sorted by treatment group is included in appendix 16.2.7. The list includes the SOC and PT MeDRA terms.

12.2.3 Analysis of Adverse Events

108 adverse events were reported thorough the study, the most frequent event reported was Headache with 14 events reported by 9 subjects (17.0%), followed by Dyspepsia with 10 events reported by 5 subjects and Urinary tract infection with 5 events reported by 3 subjects. There were no significant differences in the number of AE or the percentage of patients that reported an AE between the treatment groups. 34 were reported by 7 subjects (43.8%) in group A, 38 events reported by 10 subjects (58.8%) in group B, 10 events reported by 3 subjects (75%) in group C and 26 events reported by 11 patients (68.8%) in group X. There were no apparent trends, no relationship between dose and the number or frequency of AEs.

The most frequent events in the highest dose groups were gastrointestinal and nervous system disorders, in group A, 12 gastrointestinal events (mainly dyspepsia, diarrhoea and vomiting) were reported by 3 subjects, and 8 nervous system events (mainly headache and migraine) by 3 subjects, in both cases the percentage of subjects was 18.8%. Group B presents a similar number of events but they affected almost twice the subjects. 9 gastrointestinal events (mainly dyspepsia) were reported by 6 (35.3%) subjects and 9 nervous system events were reported by 6 (35.3%) subjects (Table 16 and Table 17).

Infections and infestations were the most affected SOC in the placebo and the low dose group (Group X). 75% of the subjects included in the placebo group (N=3) reported 3 different nervous system events, of which only one was headache; no gastrointestinal events reported in this group; however, the low number of subjects in the group impede a reliable comparison between groups or draw consistent conclusions regarding the differences found. In the low dose group (Group X) the number of subjects affected by gastrointestinal events were similar to group A, N=2 (12.5%), but the number of events, 2, were noticeably lower. In this group only one subject reported nervous system events (one headache) (Table 16 and Table 17).

System Organ Class (SOC)	Number of subjects (%) with AEs [Number of AEs]				
	Group A 3000 mg/day	Group B 1500 mg/day	Group C Placebo	Group X 500 mg/day	Total
MedDRA Preferred Term (PT)	**N=16**	**N=17**	**N=4**	**N=16**	**N=53**
Gastrointestinal disorders					
Abdominal pain upper	0(0%)[0]	1(5.9%)[1]	0(0%)[0]	0(0%)[0]	1(1.9%)[1]
Diarrhoea	1(6.3%)[1]	0(0%)[0]	0(0%)[0]	0(0%)[0]	1(1.9%)[1]
Dyspepsia	1(12.5%)[2]	1(5.9%)[3]	0(0%)[0]	0(0%)[0]	2(3.8%)[4]
Total	2(6.3%)[2]	2(11.8%)[4]	0(0%)[0]	0(0%)[0]	4(7.5%)[6]

System Organ Class (SOC)	Number of subjects (%) with AEs [Number of AEs]				
	Group A 3000 mg/day	Group B 1500 mg/day	Group C Placebo	Group X 500 mg/day	Total
MedDRA Preferred Term (PT)	N=16	N=17	N=4	N=16	N=53
Respiratory, thoracic and mediastinal disorders					
Dyspnoea	1(6.3%)[1]	0(0%)[0]	0(0%)[0]	0(0%)[0]	1(1.9%)[1]
Total	1(6.3%)[1]	0(0%)[0]	0(0%)[0]	0(0%)[0]	1(1.9%)[1]
Reproductive system and breast disorders					
Polymenorrhoea	1(6.3%)[1]	0(0%)[0]	0(0%)[0]	0(0%)[0]	1(1.9%)[1]
Heavy menstrual bleeding	1(6.3%)[1]	0(0%)[0]	0(0%)[0]	0(0%)[0]	1(1.9%)[1]
Total	1(6.3%)[2]	0(0%)[0]	0(0%)[0]	0(0%)[0]	1(1.9%)[2]

TABLE 10. FREQUENCY OF ADVERSE EVENTS POSSIBLY OR PROBABLY RELATED TO THE INVESTIGATIONAL PRODUCT.

Among the related events the most prevalent were the gastrointestinal disorders with 6 events, mainly dyspepsia (66.7% of the events), reported by 4 patients (Table 10). All related events were mild and reported by subjects included in the two highest dose groups, group A and group B. The most common event in both groups was dyspepsia with 2 and 3 events respectively, reported by one subject in each group. The remaining events were single events reported by one subject each (Table 10).

No related events occurred in the placebo group or in the low dose group.

12.2.4 Listing of Adverse Events by Patient

A thorough list of adverse events, sorted by the treatment group is included in Appendix 16.2.7 Adverse Events Listing (each subject).

12.3 Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

12.3.1 Listing of Deaths, Serious Adverse Events and Adverse Events of Special Interest

The following table details the serious adverse events that were recorded during the study.

ID	Group	AE SOC	AE PT	Start	End	Causality	Intensity	Outcome
201	X	Surgical and medical procedures	Hospitalisation	06/02/2022	11/02/2022	Unrelated	Mild	UK
204	A	Injury, poisoning and procedural complications	Road traffic accident	04/12/2021	06/12/2021	Unrelated	Severe	Recovered
204	A	Injury, poisoning and procedural complications	Lower limb fracture	04/12/2021	N/A	Unrelated	Severe	Ongoing

TABLE 11. SERIOUS ADVERSE EVENTS.

12.3.2 Deaths

No deaths occurred during the study.

12.3.3 Narratives of Deaths, Other Serious Adverse Events and Certain Other Significant Adverse Events

Two subjects reported 3 SAEs, none of which were considered related to the administration of the study product.

After 80 days of treatment, subject 201 was admitted to the hospital for 5 days due to a study on weight loss and chronic fatigue. The event (hospitalization) was considered mild and not related to the IP. Despite this event, the subject completed all the procedures of the study as per protocol

Subject 204 had a traffic accident 10 days after the beginning of the administration of the IP. As a result, the subject suffered multiple fracture in the lower limb and has to be admitted to the hospital. Treatment included open reduction and inner fixation of the fracture, orthopaedic treatment, suture with surgical staples, Enantyum 25mg/8h and enoxaparin 40 mg / 24 h. The events were considered serious and severe, but unrelated to the IP, and leaded to the withdrawal of the subject.

12.3.4 Other significant Adverse Events

In addition to subject 204, two other subjects reported AEs that, despite the fact that in these cases were not considered Serious, leaded to their withdrawal from the study.

- Subject 212: A moderate ligament sprain on the 05/03/2022 impeded the subject to attend to the third visit of the study. The event was unrelated to the administration of the study product.
- Subject 234 was withdrawn due to a mild polymenorrhoea and heavy menstrual bleeding considered possibly related to the IP. The treatment was discontinued after 68 days. Nevertheless, the subject attended to the centre, 30 days after the withdrawal and completed the study questionnaires (FSS and mMRC). Although the time when the questionnaires were completed and the less medication consumed was considered as a protocol deviation, the results were included in the efficacy

analysis. No blood extraction was done during the visit, hence there are no concentration data for pro-inflammatory or pro-resolving lipid mediators.

12.4 Clinical Laboratory Evaluation

No clinical laboratory analyses were conducted to assess the safety of the product.

12.5 Vital Signs, Physical findings and other observations related to safety

Appendix 16.2.8 shows individual data of vital signs measured through the study.

There were no treatment or dose-related trends in supine systolic and diastolic blood pressure and pulse rate, or oral body temperature. The assessments of the vital signs were always normal or were considered no clinically significant.

12.6 Safety Conclusions

There are no significant differences in the number, frequency of AEs or the percentage of patients that reported AEs, between the 4 treatment groups, and no apparent trends, no relationship between dose and the number of AEs.

There were some indications towards potential gastrointestinal effects of the IP, especially at high doses, but no solid relationship can be stablished due to overall number of participants and the low number of subjects included in the control/placebo group.

There were no SAEs nor severe events than can be attributed to the use of the product. Overall, the number and characteristics of the events reported is considered acceptable and endorse the use of the product up to the highest dose tested.

13 DISCUSSION AND OVERAL CONCLUSIONS

13.1 Efficacy

4 weeks after the beginning of the treatment there were no significant changes in the mean differences of pro-inflammatory or pro-resolution mediators neither in serum nor in plasma among the treatment groups. Only for RvD2, significant differences can be observed between groups B (1.5g/day) and C (placebo), but due to the low number of subjects included in the placebo group (N=4), these differences may be an artefact. The changes in the remaining mediators are not significant and are not apparently driven by

the administration of any dose of the IP, which hampers to draw a general conclusion of its effects. When analysing the composite variables, the ratio of the mean pro-resolution:pro-inflamatory mediators increased in all groups but in the low dose group in serum and plasma.

At week 12 there was a significant raise of the 18-HEPE serum levels in the highest dose group (A: 3mg/day) compared with the rest of the groups. Showing that the oral administration of the IP can increment the plasmatic levels of this mediator of the pro-resolution response. There was also a significant increment of 18-HEPE in the lowest dose group compared with placebo. Surprisingly no differences were found between the medium dose and the placebo; the dispersion of the measured concentrations and the low number of subjects may have obscure the differences. At the end of the study there was a significant reduction of the combined pro-inflammatory mediators in group A, compared with the placebo (C) and the 1.5g/day (B) groups, but not with the lowest dose group. However, the differences observed in this composite variable were statistically significant among the 4 treatment groups at baseline.

In plasma there was a significant raise of the 18-HEPE, 17-HDHA and Mar1 concentrations in the 3 g/day group compared with groups X and B, but only regarding Mar1 with the placebo (C) group. Groups A and X show a significant reduction of the pro-inflammatory mediators PGE2 and PGF2a compared with the placebo group.

There were no other significant or relevant changes in the individual or composite variables either in serum or in plasma that revealed a trend or any effect driven by the administration of any dose of the IP where observed.

When data are analysed intragroup, a significant increase of the 18-HEPE serum and plasma concentration occurred with all 3 doses of IP and for 17-HDHA when the two highest doses are used. These findings are consistent with absence of RvE1 and RvD4 observed in placebo group, which suggests an imbalance of RvE1 and RvD4 metabolism in Long COVID patients that is improved through the IP consumption. Regarding the 14-HDHA, although slight increases were detected in serum, there were no significant differences in any of the 3 treatment groups. These data confirmed that the oral administration or the IP can significantly increment the blood concentration of 18-HEPE and 17-HDHA which are precursors of the pro-resolution mediators RvE1, RvD1, RvD2, RvD3, RvD4, RvD5, PD1 and PDx.

When considering the 14-HDHA and 17-HDHA metabolomes:prostaglandins ratio, a slight worsening is observed at 4 weeks, both in serum and in plasma. However, at week 12 the oral administration of the IP, significantly increases the maresins:prostaglandins ratio both in serum and plasma for the 3g/day group compared to baseline. The 17-HDHA metabolome, which include resolvins and protectins, Vs prostaglandins ratio does not change significantly in plasma during the study, however, a significant increase in the ratio is observed for the 3g/day group between the 4 and 12 weeks of treatment. All these results seem to point to a slow action of the IP, whose potential activity could not yield relevant results up until 12 weeks of treatment. At week 4 the decrease in the ratios might be interpreted as a worsening in the patient's pro-resolution capabilities as a result of the

disease which is corrected after 12 weeks of treatment when 3 grams of the IP are administered orally each day. The ratio of the monohidroxilated SPMS (17-HDHA, 18-HEPE, 14-HDHA):pro-inflammatory mediators (PGE2 + PGD2 + PGF2α +TXB2 + LTB4) in serum points in the same direction. The analysis of the data also revealed that the main improvement in the ratio occurred between the weeks 4 and 12 of the treatment. In plasma, the analysis of the ratio does not lead to any clear conclusions, as the evolution looks erratic and independent of the treatment group or the evolution in the study. Overall, the oral administration of the IP seems to induce a less inflammatory and more pro-resolution phenotype in long COVID patients. Further studies are required to determine the potential clinical relevance of these findings.

With regard to clinical variables, for the Fatigue Severity Scale test and for Modified Medical Research Council (mMRC) Dyspnea Scale all groups show a tendency to improve the fatigue or dyspnea symptoms; the overall score decreased for all doses and the placebo with no apparent differences.

As the main differences in the SPMs precursors and the ratios of pro-resolving:pro-inflammatory mediators or the metabolomes:prostaglandins ratios are observed at the end of the study, 12 weeks after the beginning of the treatment, it is possible that the putative clinical manifest stations of the treatment were not observed due to the short period of follow-up. As it is foreseeable that any improvement in the subject's symptoms shall emerge after the biochemical changes that were observed in the inflammatory status of the patients.

The present study has several limitations, first, the low number of subjects in the placebo group, that makes all comparison unreliable, second, the great dispersion of the data, that present high deviations, make difficult to compare the results from the different groups and can obscure small effects exert by the IP. Finally, as can be observed by the data, the effect may not be as fast as it was expected. In fact, according to the pro-inflammatory and pro-resolution data, and the ratios calculated, appears to be a slight worsening of the inflammatory status of the patients during the first weeks of the study. It is not until the last visit, 12 weeks after the beginning of the treatment were some potential beneficial effect can be observed when the ratios 14-HDHA Metabolome:prostaglandins and 17-HDHA Metabolome:prostaglandins and monohidroxilated SPMs:pro-inflammatory were analysed. The possible clinical effect might be delayed, and the follow-up time could have been too short to detect the changes produced by the effect of the IP.

13.2 Safety

The plethora of different symptoms that are present in patients with Post COVID-19 condition represents an extra challenge when conducting analysis of the safety characteristics of a product to be used in this specific population. During the study, there were no noticeable differences in the frequency, severity or seriousness of the events observed, not a clear dose-dependence. Only the two higher dose groups have reported events that were considered related to the use of the IP, mostly gastrointestinal events,

which could point to a possible effect of the product, but due to the overall low number of subjects, especially in the control/placebo group, a clear, unambiguous correspondence cannot be stablished. It is important to notice that, other studies have used the highest dose without reporting any safety issue and that the EFSA considers that supplemental intakes of EPA and DHA combined at doses up to 5g/day, supplemental intakes of EPA alone up to 1.8 g/day, or supplemental intakes of DHA alone up to about 1 g/day do not raise safety concerns for the general population, admitting that limited data are available on the effects of long-term supplementation with omega-3 fatty acids at higher doses (EFSA, 2012).

13.3 Conclusions

- The Investigational product is safe to consume and well tolerated at doses up to 3g/day in patients with long COVID condition.
- Oral administration of PUFAs and monohidroxilated SPMs significantly increases the concentrations of 17-HDHA and 18 HEPES but not of 14-HDHA in serum and plasma.
- Administration of the IP affects lipid mediator pathways and promotes a better Monohidroxilated SPMs:Pro-inflammatory ratio
- Oral administration of 3 mg/day of the IP manages to significantly reduce the pro-inflammatory mediators in serum after 12 weeks of treatment.
- The resolution pathways are significantly increased after the administration of 3g/day of IP as it is shown by 17HDHA y 14-HDHA Vs prostaglandins ratios.
- The effect of the IP over the ratio of the mediators is delayed in time; its effect is not appreciable up to 12 weeks of treatment.
- The low follow-up time and low number of subjects included in the placebo group are the most relevant weaknesses of the study. Further research is needed to see if the biochemical changes have a real clinic effect over the symptoms of COVID-19 patients.

14 TABLES, FIGURES AND GRAPS REFERRERD TO BUT NOT INCLUDED IN THE TEXT

Variable	Intercept	B	X	AIC
EPA	0.670	0.555	0.957	488.731
DHA	0.389	0.628	0.592	465.781
DPA	0.003	0.214	0.254	569.795
ARA	0.348	0.68	0.388	603.561
HEPE18	0.000	**0.044***	**0.001***	361.445
HDHA17	0.013	0.555	0.219	423.452
HDHA14	0.034	0.944	0.472	578.971
RvE1	0.655	0.431	0.470	498.442
RvD2	0.297	0.148	0.338	252.855
RvD3	0.720	0.387	0.841	252.027
RvD1	0.091	0.624	0.094	473.900
RvD4	0.224	0.213	0.357	112.778
RvD5	0.649	0.109	0.384	586.610
Mar.1	0.576	0.645	0.491	603.754
Mar.2	0.046	0.539	0.066	520.611
PD1	0.509	0.919	0.766	413.372
PDX	0.136	0.439	0.957	562.557
LXA4	0.99	0.635	0.389	195.991
LXB4	0.208	0.239	0.33	381.570
PGE2	0.095	0.163	0.431	1124.508
PGD2	0.221	0.359	0.085	889.631
PGF2a	0.051	0.158	0.194	1110.454
TXB2	0.032	0.07	0.112	1204.758
LTB4	0.037	0.356	0.334	760.794
antinfl	0.015	0.747	0.524	355.216
proinfl	0.005	**0.040***	0.055	280.84
ratio	0.606	0.662	0.374	346.401

* $p < 0.05$

TABLE 12. P VALUES FOR THE COMPARISON WITH TREATMENT A (3G/DAY) AT WEEK 12 IN SERUM.

Variable	Intercept	A	B	X	AIC
EPA	0.664	0.546	0.994	0.956	529.707
DHA	0.421	0.651	0.557	0.617	513.827
DPA	0.003	0.216	0.478	0.257	620.493
ARA	0.341	0.676	0.161	0.382	655.279
HEPE18	0.000	**0.044***	0.120	**0.001***	393.406
HDHA17	0.012	0.547	0.657	0.211	459.013
HDHA14	0.031	0.943	0.71	0.464	628.037
RvE1	0.644	0.415	0.827	0.455	538.92
RvD2	0.307	0.156	0.815	0.348	277.518
RvD3	0.721	0.388	0.952	0.842	274.708
RvD1	0.080	0.612	0.356	0.083	512.278
RvD4	0.209	0.197	0.551	0.341	119.720
RvD5	0.644	0.103	0.975	0.376	636.576
Mar.1	0.565	0.636	0.945	0.479	654.010
Mar.2	0.041	0.528	0.924	0.059	563.638
PD1	0.513	0.92	0.329	0.769	450.896
PDX	0.125	0.426	0.434	0.955	609.144
LXA4	0.990	0.648	0.204	0.408	217.609
LXB4	0.222	0.254	0.614	0.345	418.478
PGE2	0.087	0.152	0.954	0.418	1220.123
PGD2	0.205	0.342	0.583	0.074	964.127
PGF2a	0.043	0.144	0.32	0.179	1204.179
TXB2	0.027	0.062	0.215	0.100	1306.796
LTB4	0.033	0.344	0.494	0.322	825.102
antinfl	0.014	0.744	0.925	0.519	385.501
proinfl	0.004	**0.036***	0.604	0.05	304.003
ratio	0.596	0.654	0.874	0.362	374.421

* $p < 0.05$

TABLE 13. P VALUES FOR THE COMPARISON WITH TREATMENT C (PLACEBO) AT WEEK 12 IN SERUM.

Variable	Intercept	B	X	AIC
EPA	0.782	0.846	0.832	470.003
DHA	0.289	0.477	0.611	479.085
DPA	0.030	0.725	0.606	566.182
ARA	0.151	0.524	0.808	595.197
HEPE18	0.000	0.070	**0.016***	272.541
HDHA17	0.000	0.301	**0.034***	237.646
HDHA14	0.573	0.917	0.343	351.291
RvE1	0.933	0.911	0.626	435.143
RvD2	0.548	0.498	0.317	309.74
RvD3	0.330	0.972	0.652	251.007
RvD1	0.075	0.279	0.168	285.363
RvD4	0.911	0.287	0.454	220.243
RvD5	0.184	0.660	0.273	444.547
Mar.1	0.068	**0.038***	0.175	533.615
Mar.2	0.160	0.519	0.821	445.275
PD1	0.040	0.961	0.587	354.534
PDX	0.006	0.144	0.107	461.297
LXA4	0.783	0.361	0.443	231.124
LXB4	0.061	0.251	0.179	416.727
PGE2	0.428	0.055	0.292	615.98
PGD2	0.923	0.932	0.230	716.985
PGF2a	0.077	0.100	**0.048***	585.321
TXB2	0.730	0.096	0.761	797.953
LTB4	0.735	0.402	0.784	559.94
antinfl	0.009	0.294	0.29	347.862
proinfl	0.871	0.303	0.226	248.231
ratio	0.103	0.155	0.174	326.192

* $p < 0.05$

TABLE 14. P VALUES FOR THE COMPARISON WITH TREATMENT A (3G/DAY) AT WEEK 12 IN PLASMA.

Marcador	Intercept	A	B	X	AIC
EPA	0.778	0.843	0.984	0.829	509.552
DHA	0.282	0.470	0.310	0.606	519.898
DPA	0.040	0.741	0.461	0.628	622.191
ARA	0.145	0.519	0.463	0.805	646.189
HEPE18	0.000	0.061	0.140	**0.013***	293.491
HDHA17	0.000	0.290	0.237	**0.030***	256.581
HDHA14	0.560	0.914	0.565	0.327	379.038
RvE1	0.931	0.908	0.967	0.614	470.117
RvD2	0.548	0.499	0.346	0.318	337.377
RvD3	0.318	0.972	0.810	0.644	270.92
RvD1	0.068	0.267	0.062	0.157	308.334
RvD4	0.907	0.271	0.956	0.438	236.937
RvD5	0.175	0.654	0.399	0.264	481.777
Mar.1	0.060	**0.032***	0.316	0.162	577.434
Mar.2	0.146	0.505	0.493	0.815	481.252
PD1	0.035	0.960	0.718	0.576	383.147
PDX	0.005	0.132	0.175	0.096	498.848
LXA4	0.781	0.356	0.924	0.438	250.828
LXB4	0.052	0.235	0.396	0.164	450.127
PGE2	0.413	**0.048***	0.626	0.278	667.033
PGD2	0.920	0.930	0.981	0.214	776.467
PGF2a	0.068	0.090	0.251	**0.041***	633.658
TXB2	0.722	0.086	0.954	0.753	864.674
LTB4	0.731	0.396	0.544	0.782	607.985
antinfl	0.008	0.289	0.137	0.285	378.441
proinfl	0.868	0.291	0.904	0.214	268.389
ratio	0.096	0.147	0.202	0.166	353.788

* $p < 0.05$

TABLE 15. P VALUES FOR THE COMPARISON WITH TREATMENT C (PLACEBO) AT WEEK 12 IN PLASMA.

Group A					Group B					Group C					Group X				
SOC	AEs		Subjects with AE		SOC	AEs		Subjects with AE		SOC	AEs		Subjects with AE		SOC	AEs		Subjects with AE	
	No	%	No	%		No	%	No	%		No	%	No	%		No	%	No	%
Gastrointestinal disorders	12	35.3%	3	18.8%	Nervous system disorders	9	23.68%	6	35.3%	Infections and infestations	5	50.0%	2	50.0%	Infections and infestations	7	26.9%	4	25.0%
Nervous system disorders	8	23.5%	3	18.8%	Gastrointestinal disorders	9	23.7%	6	35.3%	Nervous system disorders	3	30.0%	3	75.0%	General disorders and administration site conditions	6	23.1%	3	18.8%
Reproductive system and breast disorders	4	11.8%	1	6.3%	Respiratory. thoracic and mediastinal disorders	5	13.16%	2	11.8%	Reproductive system and breast disorders	1	10.0%	1	25.0%	Musculoskeletal and connective tissue disorders	4	15.4%	3	18.8%
Respiratory. thoracic and mediastinal disorders	2	5.9%	1	6.3%	Infections and infestations	4	10.5%	4	23.5%	Respiratory. thoracic and mediastinal disorders	1	10.0%	1	25.0%	Respiratory. thoracic and mediastinal disorders	3	11.5%	1	6.3%
Injury. poisoning and procedural complications	2	5.9%	1	6.3%	Injury. poisoning and procedural complications	3	7.9%	3	17.6%						Gastrointestinal disorders	2	7.7%	2	12.5%
Musculoskeletal and connective tissue disorders	2	5.9%	1	6.3%	Psychiatric disorders	3	7.89%	2	11.8%						Surgical and medical procedures	1	3.8%	1	6.3%
General disorders and administration site conditions	2	5.9%	1	6.3%	Musculoskeletal and connective tissue disorders	2	5.26%	2	11.8%						Nervous system disorders	1	3.8%	1	6.3%
Infections and infestations	1	2.9%	1	6.3%	Vascular disorders	1	2.6%	1	5.9%						Injury. poisoning and procedural complications	1	3.8%	1	6.3%
Skin and subcutaneous tissue disorders	1	2.9%	1	6.3%	Skin and subcutaneous tissue disorders	1	2.63%	1	5.9%						Vascular disorders	1	3.8%	1	6.3%
					Immune system disorders	1	2.63%	1	5.9%										

TABLE 16. FREQUENCY OF ALL AES ORDERED BY SOC AND SORTED BY TREATMENT GROUP.

Group A					Group B					Group C					Group X				
Preferrerd Term	AEs		Subjects with AE		Preferrerd Term	AEs		Subjects with AE		Preferrerd Term	AEs		Subjects with AE		Preferrerd Term	AEs		Subjects with AE	
	No	%	No	%		No	%	No	%		No	%	No	%		No	%	No	%
Headache	5	14.7%	2	12.5%	Dyspepsia	7	18.4%	4	23.5%	Urinary tract infection	3	30.0%	1	0.25	Pain in extremity	3	11.5%	2	12.5%
Diarrhoea	3	8.8%	2	12.5%	Headache	7	18.4%	5	29.4%	Amnesia	1	10.0%	1	0.25	Bronchitis	2	7.7%	1	6.3%
Dyspepsia	3	8.8%	1	6.3%	Amnesia	2	5.3%	1	5.9%	Dysmenorrhoea	1	10.0%	1	0.25	Covid-19	2	7.7%	2	12.5%
Migraine	3	8.8%	1	6.3%	Disorientation	2	5.3%	1	5.9%	Gastroenteritis	1	10.0%	1	0.25	Inflammation	2	7.7%	1	6.3%
Vomiting	3	8.8%	1	6.3%	Oropharyngeal pain	2	5.3%	1	5.9%	Headache	1	10.0%	1	0.25	Pain	2	7.7%	2	12.5%
Asthenia	2	5.9%	1	6.3%	Urinary tract infection	2	5.3%	2	11.8%	Nasal congestion	1	10.0%	1	0.25	Rhinitis allergic	2	7.7%	1	6.3%
Dysmenorrhoea	2	5.9%	1	6.3%	Abdominal pain upper	1	2.6%	1	5.9%	Nasopharyngitis	1	10.0%	1	0.25	Arthralgia	1	3.8%	1	6.3%
Dyspnoea	2	5.9%	1	6.3%	Campylobacter gastroenteritis	1	2.6%	1	5.9%	Sciatica	1	10.0%	1	0.25	Catarrh	1	3.8%	1	6.3%
Arthralgia	1	2.9%	1	6.3%	Foot fracture	1	2.6%	1	5.9%						Diarrhoea	1	3.8%	1	6.3%
Covid-19	1	2.9%	1	6.3%	Frequent bowel movements	1	2.6%	1	5.9%						Duodenogastric reflux	1	3.8%	1	6.3%
Duodenogastric reflux	1	2.9%	1	6.3%	Haematoma	1	2.6%	1	5.9%						Fall	1	3.8%	1	6.3%
Flatulence	1	2.9%	1	6.3%	Insomnia	1	2.6%	1	5.9%						Fatigue	1	3.8%	1	6.3%
Heavy menstrual bleeding	1	2.9%	1	6.3%	Ligament sprain	1	2.6%	1	5.9%						Headache	1	3.8%	1	6.3%
Hyperhidrosis	1	2.9%	1	6.3%	Musculoskeletal pain	1	2.6%	1	5.9%						Herpes zoster	1	3.8%	1	6.3%
Lower limb fracture	1	2.9%	1	6.3%	Nasal congestion	1	2.6%	1	5.9%						Hospitalisation	1	3.8%	1	6.3%
Myalgia	1	2.9%	1	6.3%	Pain in extremity	1	2.6%	1	5.9%						Influenza	1	3.8%	1	6.3%
Nausea	1	2.9%	1	6.3%	Pain of skin	1	2.6%	1	5.9%						Pyrexia	1	3.8%	1	6.3%
Polymenorrhoea	1	2.9%	1	6.3%	Post-traumatic pain	1	2.6%	1	5.9%						Raynaud's phenomenon	1	3.8%	1	6.3%
Road traffic accident	1	2.9%	1	6.3%	Respiratory tract infection	1	2.6%	1	5.9%						Respiratory tract infection	1	3.8%	1	6.3%
					Rhinitis allergic	1	2.6%	1	5.9%										
					Rhinorrhoea	1	2.6%	1	5.9%										
					Seasonal allergy	1	2.6%	1	5.9%										

TABLE 17. FREQUENCY OF ALL AES ORDERED BY PT AND SORTED BY TREATMENT GROUP.

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16 APPENDICES