Internal Medicine Department
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The role of VSL#3® in the treatment of fatigue and other symptoms in Long COVID 19 syndrome: a randomized, double-blind, placebo-controlled study

Acronym: DELong#3/2022
Protocol version number: v.1.0
Date: 14/05/2022

Sponsor: Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico
Address: Via Sforza 28, 20122 Milan, Italy

Coordinating Center: Gastroenterology and Endoscopy Unit
Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico
Address: Via Francesco Sforza 35, 20122 Milan, Italy

Principal Investigator: Prof. Flavio Andrea Caprioli

<Firma>_____

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FLOWCHART

	Clinical Trial			
	Screening	Randomization	Procedure	Follow-up
Period	Visit 1 (-2t)	Visit 2 (t0)	Visit 3 (t4)	Visit 4 (t8)
Enrollment				
Informed consent	X			
Inclusion/exclusione criteria	X			
Procedures				
VSL#3®		X	X	
Placebo		X	X	
Assessments				
[stool and blood sample]	X	X		
[primary endpoint]		X	X	X
[secondary endopoint]		X	X	X

t=week

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ABBREVIATION LISTS

CCL: Chemokine Ligand

EC: Ethical Committee

IC: Informed Consent

ChFS: Chalder Fatigue Scale

CFS/ME: Chronic Fatigue Syndrome / Myalgic Encephalomyelitis

CRF: Case Report Form

CSF: Colony Stimulating Factor

CXCL: C-X-C Motif Chemokine Ligand FGF:

Fibroblast Growth Factor

FLTL3: FMS-like Tyrosine Kinase 3

Ligand FM: Fibromyalgia

GCP: Good Clinical Practice

GRZ: Granzyme

IBS: Irritable Bowel Syndrome

IEO: Institute Europeo of Oncology

IFN: Interferon

IL: Interleukin

MERS: Middle-East Respiratory Syndrome

PDGF: Platelet-Derived Growth Factor

SARS: Severe Acute Respiratory Syndrome

TGFA: Transforming Growth Factor Alpha

TNF: Tumor Necrosis Factor

TRAIL: Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand

VEGF: Vascular Endothelial Growth Factor

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1.RESPONSABILITIES (role of the sponsor and collaborators)

The sponsor of the study is Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico. The coordinating center will be the UOC of Gastroenterology and Endoscopy at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico.

The role of the Principal Investigator (PI) will be fulfilled by Prof. Flavio Andrea Caprioli, UOC of Gastroenterology and Endoscopy - Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico. The Principal Investigator will be responsible for patient recruitment, evaluation of inclusion and exclusion criteria, data acquisition, and interpretation of the data.

Dr. *Guido Basilisco* - study concept and design

Dr. *Dario Consonni* - statistical analyses

Internal collaborations

Operating Unit	Participant Name	Role and Functions in the Study
Gastroenterology and Endoscopy Unit	*Dott. Guido Basilisco*	*Conceptualization*
Occupational Medicine Unit	*Dott. Dario Consonni*	*Statistical Analysis*
Infectious Diseases Unit	*Prof. Andrea Gori*	*Identification of eligible patients to the PI*
Pneumology Unit	*Prof. Blasi*	*Identification of eligible patients to the PI*

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External collaborations (biological sample analysis, data analysis, diagnostic procedures, etc.)

Istitution	*Operating Unit*	*Paticipants name*	*Role and Functions in the study*
Istituto Europeo di Oncologia	*Mucosal Immunology Laboratory*	*Dott.ssa Federica Facciotti*	*Immunological and microbiological analyses*

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1. INTRODUZIONE

1.1 Background

Viral, bacterial, and protozoal infections are associated with a diverse clinical spectrum of symptoms, including fatigue, difficulty concentrating, depression, sleep disturbances, gastrointestinal disorders, and musculoskeletal pain [1-3]. In previous coronavirus epidemics such as Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS), studies have linked the infection to the possible development of Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) [4-6]. In particular, a study by Lam et al. conducted on 233 SARS survivors revealed that after 4 years, 27.1% of patients met the criteria for a CFS/ME diagnosis [7]. In the field of gastroenterology, several studies have demonstrated that previous infections of the gastrointestinal tract can lead to Irritable Bowel Syndrome (IBS) and CFS/ME. A study conducted on a Norwegian population of 576 individuals followed for 10 years after a Giardia Lamblia epidemic showed that the relative risk of developing IBS was 4.74 compared to an uninfected control group, while the relative risk of CFS/ME was 3.01 [8]. Another significant German study involving a large number of patients (508,278) found an increased relative risk of IBS and CFS/ME (1.73 and 2.08, respectively) in patients diagnosed with infectious gastroenteritis [9]. Several mechanisms may be involved in the pathogenesis of CFS and IBS associated with previous infection. Among these, it is possible that viral, bacterial, or protozoal infections lead to a modification of the intestinal microbiota in qualitative and quantitative terms. This alteration could result in a pro-inflammatory phenotype, leading to the release of bacterial toxins and inflammatory cytokines into the systemic circulation through areas of altered intestinal permeability (leaky gut) [10-17]. The same alteration of intestinal permeability and the microbiota may also be involved in various neurological and psychiatric disorders reflecting an alteration of the so-called "brain-gut axis" [11,18-20]. Additionally, cognitive impairment may play a role in the perception of fatigue. Persistent cognitive impairments have been documented following SARS-CoV-2 infection [21-23],

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and a recent Italian study on a group of 38 hospitalized COVID-19 patients demonstrated deficits in processing speed and verbal memory [23]. Several studies have attempted to intervene in the dysbiotic intestinal pathogenic mechanism of CFS/ME. A systematic review with meta-analysis from 2018 analyzed the use of probiotics in the context of CFS/ME and IBS in 25 studies. The possible therapeutic role of probiotics is recognized, but the low quality of the trials, overall lack of evidence, and the different composition of these products do not allow for the affirmation of their actual effectiveness [24]. Similar conclusions are reported in recent American guidelines on IBS, which confirm the role of infections and the microbiota in the pathogenesis of this disease but also lament the poor quality of evidence (mostly based on monocentric studies with small sample sizes) to recommend their systematic use [25]. The same guidelines cite a meta-analysis by Ford et al. from 2014, which summarizes the results of 37 randomized clinical trials in IBS and concludes that probiotics have been associated with improvements in overall symptomatology, abdominal pain, bloating sensation, and flatulence [26]. However, the authors themselves declare that the evidence of probiotic effects is influenced by the different composition of commercially available products and the poor quality of the conducted studies [26].

During the current COVID-19 epidemic, a series of scientific studies have demonstrated an alteration in the composition of the intestinal microbiota during SARS-CoV-2 infection [27-29]. This alteration, compared to the gut flora of healthy controls, appears to persist beyond the hospitalization period, be independent of the administered therapy, be associated with the presence of inflammatory taxa, and be more extensive and radical than that induced by H1N1 influenza infection [27-29].

Furthermore, starting from the first wave of COVID-19, a new post-infectious syndrome called "Long-Covid19 Syndrome" has been described in several countries, characterized by the persistence of clinical symptoms after recovery from COVID-19 according to WHO criteria [30,31]. Carfì et al. analyzed 143 hospitalized patients who were discharged after an average of 60 days from the onset of the disease and found that up to 87.4% of patients had at least one persistent

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symptom, with fatigue being the main one (53.1%) [32]. In a study conducted using an online platform, Goërtz et al. demonstrated that in a cohort of 2113 patients (including patients with a confirmed or suspected diagnosis of COVID-19), the number of symptoms experienced 79 days from the onset of the illness had significantly reduced, but at the same time, only 0.7% of patients were symptom-free. Once again, fatigue was the most reported symptom (87%) [33]. A study by Townsend et al. from Ireland, involving 128 patients, approximately half of whom were hospitalized for COVID-19, evaluated after a median time of 72 days, showed that only 42% of these patients had a health status comparable to that before the infection. 52% of the subjects met the diagnostic criteria for fatigue, with Chalder Fatigue Scale (ChFS) scores similar to those of patients with CFS/ME. Surprisingly, the state of fatigue does not seem to be correlated with the clinical severity of SARS-CoV-2 infection or the presence of inflammatory changes (e.g., white blood cell count, neutrophil count, neutrophil-to-lymphocyte ratio, and levels of PCR, LDH, IL-6), suggesting a more subtle etiopathogenic mechanism in line with other observations in CFS/ME [34]. Another Norwegian study observed the prevalence of fatigue in a cohort of non-hospitalized SARS-CoV-2 positive patients, using the ChFS via postal survey conducted at a distance from the diagnosis of COVID-19. The 458 enrolled patients responded after a median time of 117 days from SARS-CoV-2 infection, and 46% showed a ChFS score (in dichotomous mode) compatible with clinically significant fatigue, while the Likert Scale score of the same scale differed significantly from that obtained from a cohort of healthy subjects [35]. Finally, in our own case series of 164 patients studied five months after SARS-CoV-2 infection, compared to a control group of 183 subjects, the relative risk of CFS was increased by 2.24.

VSL#3® is a patented high-concentration blend (450 billion bacterial units) of live and lyophilized lactic acid bacteria and bifidobacteria, belonging to eight different strains (one strain of Streptococcus thermophilus BT01; three strains of Bifidobacteria: B. breve BB02, B. animalis subsp. lactis BL03 (formerly known as B. longum BL03), and B. animalis subsp. lactis BI04 (formerly known as B. infantis BI04); four strains of Lactobacilli: L. acidophilus BA05, L.

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plantarum BP06, L. paracasei BP07, and L. helveticus BD08 (formerly identified as L. delbrueckii subsp. bulgaricus BD08)).

Animal studies support the biological plausibility that modulation of the microbiome can affect perception and cognitive-behavioral mechanisms [36]. In an exploratory study in patients with IBS, Bifidobacterium Longum reduced depression scores and improved the patients' quality of life. These improvements were associated with changes in brain activation patterns indicating that the probiotic reduces limbic reactivity [37]. Recently, a comparative study of VSL#3 versus a LOW-FODMAP diet was published, showing that both treatments had fundamentally the same efficacy [38].

2. STUDY OBJECTIVE/HYPOTHESIS

2.1 Primary Objectives

The primary objective of this study is to test whether a high-concentration probiotic formulation (e.g., VSL#3®) can reduce the degree of clinically significant fatigue in ambulatory patients suffering from Long-Covid-19 Syndrome, i.e., the persistence of systemic symptoms 4 weeks after molecular detection of SARS-CoV-2

2.2 Secondary Objective(s)

1.To determine if this high-concentration probiotic formulation can improve scores of anxiety, depression, quality of life, gastrointestinal symptoms, cognitive deficits, performance, psychological distress, and clinical evaluation of the study patients.

2.To assess the immunological profile of inflammation markers in blood and bacterial species in feces in patients treated with VSL#3® compared to patients treated with a placebo. Any differences before and after treatment will be identified.

3.STUDY DESIGN

3.1 Study Design

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Our study is a double-blind, randomized, placebo-controlled trial. Anticipating a higher representation of female subjects in our sample, we hypothesized a stratified block randomization by sex. This is a single-center study conducted in an outpatient setting, and currently hospitalized patients will not be recruited.

3.2 Inclusion Criteria

Patients who meet all of the following criteria will be included:

- Ambulatory patients who have signed informed consent.
- Age above 18 years and below 65 years.
- Previous diagnosis of SARS-CoV-2 infection, documented by molecular nasopharyngeal or antigen swab.
- Not currently undergoing isolation or quarantine.
- Not having used antibiotic products within the 30 days preceding the trial.
- Chalder Fatigue Scale (in dichotomous form) >=4, possibly associated with signs and symptoms of Long-Covid-19 Syndrome [31,32]: signs and symptoms that develop during or after SARS-CoV-2 infection, persist for more than 4 weeks, and are not reasonably explained otherwise; signs and symptoms include fatigue, sleep disturbances, cognitive deficits (i.e., brain fogging, loss of concentration and memory, anxiety, depression), muscle weakness, joint pain, gastrointestinal alterations (reduced appetite, nausea, changes in bowel habits such as constipation and/or diarrhea, abdominal pain)

3.3 Exclusion Criteria

Patients with at least one of the following criteria will be excluded:

- Moderate to severe cardiovascular and pulmonary diseases with organ dysfunction (NYHA>2, Borg scale >=2).
- Decompensated endocrine and metabolic diseases (Child-Pugh cirrhosis >= B, decompensated hypo/hyperthyroidism, decompensated adrenal insufficiency).
- Pre-existing diagnosis of FM, CFS/ME, and/or IBS prior to SARS-CoV-2 infection.

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- Confirmed diagnoses of neurological disorders, psychiatric illnesses, and cognitive impairments prior to SARS-CoV-2 infection.
- Previously confirmed diagnoses of chronic musculoskeletal disorders prior to SARS-CoV-2 infection.
- Refusal to participate in the study / refusal to allow the processing of personal data.
- Pregnancy or breastfeeding.
- Alcohol or drug dependency in the previous years.
- Use of other probiotics during the trial.
- Use of antibiotics during the trial and in the 30 days preceding it.
- Substantial change in diet during the trial.
- Participation in another clinical study in the 30 days preceding or previous participation in the same trial.
- Known intolerance/hypersensitivity to the investigational product or the excipients of the placebo formulation.

3.4 Study Withdrawal Criteria

- Onset of hypersensitivity to the study product or the placebo
- Clinical necessity for the use of antibiotics, immunosuppressants or immunomodulators, glucocorticoids, opioids, antidepressants, or anxiolytics at regular and continuous dosages.

4. PROCEDURES RELATED TO THE STUDY

4.1 Intervention Patients

Patients will be referred by the Pneumology and Infectious Diseases outpatient clinics for a preliminary evaluation of inclusion and exclusion criteria, within the context of scheduled visits for sequelae of SARS-CoV-2 infection. The recruitment phase is divided into an initial screening visit (Visit 1, t-2) to determine if the patient meets the inclusion/exclusion criteria. Following this visit, a

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14-day run-in period will be conducted to screen for non-adherent patients, i.e., those who do not show up for the subsequent visit. The patient will return for a visit at 14 days (Visit 2, t0) when randomization will occur, and the patient will be assigned to the treatment or placebo group. The patient will be instructed to take the treatment (supplement or placebo) at the prescribed dosage of two sachets per day for a consecutive and continuous period of four weeks. During this visit, all immunological and microbiological analyses will be performed, including the collection of 20 mL of blood and 1 g of fecal material. The patient will also complete clinical questionnaires to assess the primary and secondary endpoints. The clinical questionnaires will be stored in a deidentified form at the Gastroenterology and Endoscopy Unit, while the biological samples will be shipped and analyzed at the Mucosal Immunology Laboratory of the IEO. Considering the short duration of the study and the lack of literature data demonstrating a clear benefit of VSL#3 on this syndrome, no crossover between study arms is planned under any circumstances. During the study, the use of immunosuppressants or immunomodulators, glucocorticoids, opioids, antidepressants, or anxiolytics at regular and continuous dosages is not allowed. If there is a clinical necessity for antibiotic therapy, it will result in discontinuation of the study. After completing the 28-day treatment period, the patient will return for a visit (Visit 3, t4) where immunological and microbiological analyses will be repeated, and questionnaires will be completed again to evaluate the primary and secondary endpoints. An additional visit with completion of clinical questionnaires will be scheduled 28 days after the end of the treatment (Visit 4, t8) to assess whether any response is maintained at the end of the treatment period.

4.2 Randomization

Randomization will be managed using the REDCap (Research Electronic Data Capture) software. Considering the relatively small number of anticipated patients (n = 96, see dedicated section), we have planned a permuted block randomization with a 1:1 ratio between the placebo and treatment groups. Given the higher expression of the Long-Covid-19 Syndrome in females, randomization will be stratified by sex. We do not anticipate any socio-health or ethical factors that could interfere

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with the randomization process to bias patients into one arm over another.

4.3 Blinding

The study participants, healthcare professionals delivering the intervention, and outcome assessors will be blinded to the treatment being administered. Actial Farmaceutica will provide drug and placebo packages that are indistinguishable from each other and will disclose the nature of the two treatments at the end of the study analyses. In the event of serious adverse effects that require hospitalization and the patient's admission, an external collaborator not involved in the study will be informed of the treatment nature through direct contact with Actial Farmaceutica to intervene if necessary.

5. ENDPOINTS

5.1 Primary Endpoint

Demonstration of a statistically significant difference between the placebo group and the treatment group in the scores of the Chalder Fatigue Scale at the end of the treatment period (t4). The Chalder Fatigue Scale is a validated scale used to assess fatigue in the context of CFS/ME [39-41].

5.2 Secondary Endpoints

Demonstration of a statistically significant difference between the placebo group and the treatment group after 4 weeks of treatment (t4) and after an additional 4-week follow-up period (t8; 1 month after the end of treatment), unless otherwise specified, for the following variables:

- Scores of the Chalder Fatigue Scale (t8)
- Hospital Anxiety and Depression Scale (HADS). HADS is a validated scale that quantifies anxiety and depression experienced by the patient [42,43].
- Short Form Health Survey (SF-36). SF-36 is a validated scale that assesses health-related quality of life [44].
- Structured Assessment of Gastrointestinal Symptoms Scale (SAGIS). SAGIS is a validated scale that measures the intensity and impact of gastrointestinal symptoms [45].

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- Somatic symptoms of the Symptom Checklist-90 (SCL-90) assessed by the SCL 12. SCL-90 is a validated questionnaire that assesses psychological distress in individuals [46-52,54].
- Karnofsky Performance Status (KPS) Scale. KPS is a validated scale consisting of 11 points that describes the overall functional status of a patient on a range from 100% (no evidence of disease, no symptoms) to 0% (death) [49,50,51,52].
- Visual Analog Scale (VAS) rating of the patient's overall health status by the examining physician.
- Serum expression of cytokines (IL-2, IL-7, IL-15, IL-10, IL-33, IL-4, IL-5, IL-13, IL-1a, IL-1b, IL-1Ra, IL-12p70, IFNa, IFNb, IFNg, TNFa, TRAIL, GRZb, IL-6, IL-17A, IL-17F, IL-22, and IL-23), chemokines (CXCL1 (GROa), CXCL2 (GROb), CXCL8 (IL-8), CXCL10 (IP-10), CX3CL1 (Fraktalkine), CCL2 (MCP-1), CCL3 (MIP1a), CCL4 (MIP-1b), CCL5 (Rantes), CCL11 (Eotaxin), CCL19 (MIP3b), and CCL20 (MIP3a)), growth factors (FLT3L, IL-3, G-CSF, GM-CSF, TGFa, EGF, PDGFaa, PDGFab/bb, VEGF, and FGF basic), and immune activation markers (CD40L and PDL1).
- Variation in the bacterial component of the fecal microbiota in terms of alpha and beta diversity and correlation of these parameters with clinical response on fatigue.

6. STUDY DURATION / TIMELINE

The study will have a total duration of 70 days per enrolled patient. Please refer to the attached Gantt chart.

Study Start: 09/2022 Recruitment Closure: 12/2022 Study End: 07/2023

7.STATISTICAL ANALYSIS

7.1 Sample Size

Assuming a placebo effect of 30% reduction in the primary outcome (fatigue assessed by Chalder Fatigue Scale - Likert 0-33), a statistical power of 80% with a significance level of 0.05 (two-tailed),

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we estimate that 42 patients per arm are needed to detect a 60% efficacy of the probiotic in reducing the same parameter. This reduction, if confirmed, would have significant clinical relevance, indicating a substantial efficacy compared to placebo. Considering a dropout rate of 15%, we believe that recruiting 48 actual patients per arm is necessary to demonstrate this efficacy.

7.2 Data Analysis

Both intention-to-treat and per-protocol analyses will be conducted. Depending on the distribution of the data, Student's t-test or Wilcoxon Mann-Whitney test will be used for continuous variables, and Chi-square test or Fisher's exact test for categorical variables. Relationships between multiple variables will be assessed using linear or logistic regression tests based on the nature of the available data. Statistical analysis will be performed using the RStudio software (R Core Team, 2020) and Stata software (StataCorp, 2013). No interim analyses are planned due to the short duration of the study and the relatively small number of enrolled patients.

8.ADVERSE EVENTS

The project does not involve the administration of drugs or other substances or invasive clinical procedures. Therefore, no significant adverse events are expected, except those related to venous blood collection. Serious adverse effects associated with the ingestion of the studied probiotic are not reported in the literature, except for a temporary increase in the sensation of abdominal bloating following the initial doses of the probiotic.

9. RISK / BENEFIT ASSESSMENT

Currently, there is no established cure for Long-Covid-19 Syndrome. The use of a probiotic formulation offers the advantage of an excellent safety profile compared to pharmacological formulations. However, since the pathogenic mechanism of this syndrome is unknown, the benefit of the probiotic formulation cannot be established a priori. A follow-up of the patients will be conducted one month after the end of the study, primarily to assess the persistence of any potential

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benefits rather than the occurrence of adverse events.

10. STUDY MANAGEMENT

10.1 Data Collection and Management

The study data will be collected using an electronic Case Report Form (CRF) managed through REDCap (Research Electronic Data Capture) software. A supplementary module will be available for the investigator to enter patient demographic data, SARS-CoV-2 infection information, and the results of the study questionnaires. The questionnaires to be used in the study, along with their respective references, are listed in the primary and secondary outcome sections. Immunological and microbiological investigation data will be uploaded in electronic format. Patient information will be entered in a pseudonymized manner into a database managed exclusively by the investigator or designated collaborators, with each patient identified by a unique code. The file linking the participant code with their identifying data will be kept separately on a password-protected computer. The study database will be password-protected and stored on a computer accessible only to authorized study personnel designated by the principal investigator. Deidentification of data will be done in a way that those accessing the database cannot link the data to the participants' identities. Only local investigators will have access to the identities of enrolled participants.

10.2 Regulatory and Ethical Considerations

10.2.1 Approval from Competent Authority

In accordance with applicable regulations, the principal investigator must obtain approval from the appropriate Competent Authority before commencing the clinical study. This study will be conducted in compliance with the rules of ICH/GCP (International Conference of Harmonization/Good Clinical Practice) and all applicable laws, including the Declaration of Helsinki of June 1964, modified by the latest World Medical Association General Assembly in Seoul, 2008.

10.2.2 Ethical Committee Approval

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The investigator must ensure that the protocol has been reviewed and approved by the local independent Ethics Committee (EC) before commencing the study. The EC must also review and approve the Informed Consent (IC) form and all written information received by the patient prior to enrollment in the study. If it becomes necessary to modify the protocol and/or IC during the study, the investigator will be responsible for ensuring the review and approval of the modified document by the EC. The content of such modifications will be implemented only after approval by the EC. Until then, the previously approved version of the document must be referred to.

10.2.3 Informed Consent (IC)

The investigator or authorized personnel must inform individuals about all aspects and procedures of the study. The investigator or authorized personnel will obtain informed consent or assent from potential trial participants or their legal representatives. The process of obtaining informed consent must comply with current regulatory procedures. The investigator (or a designated collaborator) and the subject must date and sign the IC form before the patient undergoes any study-related procedures. The subject will receive a dated and signed copy of the IC form from both parties; the original copy will be retained in designated study archives. Neither the investigator nor the designated personnel should in any way coerce or unduly influence a subject to participate or continue participating in the study. The subject's decision to participate in the study must be completely voluntary. The investigator and designated personnel must emphasize to the subject that they may withdraw their consent at any time without penalty or loss of any entitled benefits. Written or oral information regarding the study, including the written IC form, must not contain any language that would require the subject to waive (even apparently) their legal rights or that would release the investigator, institution, or sponsor from liability for negligence.

10.3 Investigator's Responsibilities

In accordance with applicable local regulations, the investigator must provide periodic reports regarding the progress of the study at their center to the EC and notify the EC of the study's closure. Periodic reports and closure notification are part of the investigator's responsibilities.

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10.4 Study Monitoring

In accordance with applicable regulations and Good Clinical Practice (GCP), the monitor must periodically visit or contact the study site. The duration, nature, and frequency of these visits/contacts depend on the recruitment rate, the quality of the documents held by the site, and their adherence to the protocol.

Through these contacts, the monitor must:

- Monitor and evaluate the progress of the study
- Review the collected data
- Perform source data verification
- Identify any issues and propose solutions

The purposes of the monitoring activities are to ensure that:

- The rights and well-being of the subjects are protected
- The study data is accurate, complete, and verifiable from the original documents
- The study is conducted in compliance with the protocol, approved amendments, GCP, and applicable regulations

The investigator must:

- Provide the monitor with direct access to all relevant documentation
- Dedicate time and personnel to meet with the monitor to discuss the monitoring results and address any other relevant aspects.

The monitor should also contact the site prior to the study initiation to discuss the protocol and data collection procedures with the staff.

10.5 Study Quality Assurance

As the sponsor, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico may, at its discretion, perform quality control of the study. In this case, the investigator must allow the monitor direct access to all relevant documentation and dedicate time and personnel to discuss the monitoring results and any other aspects of the study. Additionally, Regulatory Authorities may

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conduct inspections. In such cases, the investigator must authorize the inspector's direct access to all relevant documentation and dedicate time and personnel to discuss the monitoring results and any other aspects of the study.

10.6 Study Closure

At the time of study closure, the monitor and investigator must initiate a series of procedures, including:

- Reviewing all study documentation
- Reconciling study data
- Resolving any outstanding queries

10.7 Document Archiving

In accordance with applicable national regulations, the investigator must retain a copy of all documentation in a dry and secure location after study closure.

10.8 Disclosure of Scientific Findings 10.8.1 Confidentiality The investigator and other personnel involved in the study must treat all study-related information (including the protocol, obtained data, and all study-related documentation) confidentially and must not use such information, data, or findings for purposes other than those described in the protocol. These restrictions do not apply to:

1. Information that becomes publicly available without negligence on the part of the investigator or their personnel
2. Information that requires confidential disclosure to the EC solely for the purpose of evaluating the study
3. Information that needs to be disclosed to obtain appropriate medical care for a study subject.

10.8.2 Publications

Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico is the sole owner of the data.

10.9 Intellectual Property Rights on Study Results

If, during the project, know-how, technical materials, and/or goods protected by industrial and/or intellectual property rights or susceptible to protection are used and shared to the extent strictly

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necessary for the conduct of the project, developed by the Promoter or the collaborating center ("Background"), it is understood that such background will remain the property of the party that developed it, and the use or sharing of it by the owning party will not result in the acquisition of any rights by the other party. The results and data generated during the study will remain the exclusive property of the Promoter, who agrees to grant the Collaborating Center the right to use them solely for internal research activities after publication by the Promoter.

11.COMPENSATION AND DAMAGES IN CASE OF HARM

In the event of adverse events or potential damages resulting from participation in the research, the Insurance Policy of our Institute extends coverage to the participating subjects in research projects.

12. PROTOCOL AMENDMENTS

Not applicable.

13. FINANCIAL AGREEMENTS

This study is funded by Actial Farmaceutica Srl - Viale Shakespeare 47 00144 Rome (Italy), as specified in the attached funding agreement.

14. DISCLOSURE OF CONFLICTS OF INTEREST

The authors of this protocol declare no conflicts of interest.

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