Clinical Trial Protocol

Coenzyme Q10 as treatment for Long Term COVID-19
(The QVID study)

An investigator-initiated randomized, placebo-controlled, phase IIa trial

Trial Identification: QVID-001
EudraCT No: 2020-005961-16

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1.6 Guidelines

This study will be conducted in accordance with the protocol, The Helsinki Declaration (1996 version), The International Conference on Harmonization guidelines for GCP and national ethical guidelines and law.

1.7 Time plan

We plan to enroll participants from February 1st 2021 until December 31st 2021. The last planned visit date will be in 2021. Expected final report by end 2022.

1.8 Signatures Page 1

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable European Union regulations and The International Conference on Harmonization guidelines.

The Sponsor, Lead Principal Investigator and Coordinating Investigator should sign Signature Page 1. A copy of this Signature Page 1 should be filed with the holder of the Regulatory documents and a copy should be maintained at each site.

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2 Introduction

2.1 Background and Rationale

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was discovered as an outbreak in Wuhan, China in 2019 and has since spread across the globe, creating a pandemic. SARS-CoV-2 causes the disease new-coronavirus-disease-2019 (COVID-19)[1]. Currently large parts of Europe and The United States are experiencing high numbers of new infections, leading to overwhelmed health care facilities and concomitant lock-downs of societies in order to contain the virus.[2] Clinical COVID-19 research has inherently been occupied with treatment of the acute infection, critical COVID-19 illness, test-strategies, immunity and vaccines. An increasing number of reports do however suggest, that a small proportion of persons recovering from the acute infection suffer from persistent symptoms such as abdominal pain, diarrhea, chest pain, unusual myalgia, paresthesia in hands and feet, intermittent fever, persistent cough, loss of smell and taste, sleep disturbances, shortness of breath, headache, fatigue, cognitive/psychological problems and mood disturbances.[34] This phenomenon is also named ”Long Term COVID-19”, ”post-COVID-19 Syndrome” or ”Long Haulers”.[3–5]

Similar prolonged debilitating symptoms such as muscle pain, sleep disturbances and fatigue were also observed in patients recovering from SARS-1 (2002–2003). In some cases, symptoms persisted for years and patients never returned to work after the acute illness.[6] Long Term COVID-19 (LTC) therefore has the potential to become a persistent and severe condition both for individuals and societies.

In patients with the condition Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFE), a similar symptomatology is reported, including diffuse symptoms from several organ system: affected cognitive functions, post-extertional malaise, pain, sleep disturbances, palpitations and aberrant immune activation.[7,8] The etiology of ME/CSF is unknown, but altered mitochondrial function is found in several studies.[9] Preliminary unpublished data and evidence from this partly comparable chronic fatigue condition points towards a cellular metabolic disturbance as part of the pathogenesis for LTC. Specifically, it is plausible that LTC is associated with increased cellular *anaerobe* metabolism resulting in decreased energy production and increased oxidative stress. This assumption corresponds to the symptomatology reported, as described above.[5]

Coenzyme Q10 (CoQ10) – also known as ubiquinone – is part of the electron transport chain, responsible for the ATP synthesis in the mitochondria. It is an electron transporter and is involved in oxidative phosphorylation (OXPHOS) in the inner mitochondrial membrane. CoQ10 is the only molecule with these attributes and is thus essential for cellular *aerobe* metabolism. Further, it functions as an antioxidant and is constantly in a oxidation-reduction-cycle, hereby reducing the levels of free radicals.[10,1112] Studies have shown that supplementary CoQ10 can reduce oxidative stress and reestablish normal mitochondrial function.[11]

It has been shown that ME/CSF patients has significantly lower plasma levels of CoQ10 and severity of fatigue symptoms correlates with CoQ10 levels.[13] In a clinical trial with ME/CSF patients, a significant improvement in fatigue was observed in patients after 8 weeks of CoQ10 supplementation compared to placebo.[14]

Given the similarities between LTC and ME/CSF regarding proposed pathophysiology and clinical presentation it is possible, that CoQ10 supplement will improve cellular metabolism and reduce symptoms in LTC, as proposed in a recently published review.[15]

In summary, we aim to investigate if high-dose CoQ10, can reduce the number and severity of symptoms related to LTC.

We hypothesize that LTC-related symptoms are caused by reduced capacity for cellular OXPHOS and that high-dose supplements will significantly decrease number and severity of symptoms in LTC patients.

3 Overview of the Investigational Medicines Products (IMPs)

3.1 Coenzyme Q10 (CoQ10) Supplement

3.1.1 Experience and Dosing

CoQ10 is categorized as a dietary supplement in the United States and Europe, while in Japan and Europe it is labeled as a medicines product for patients with heart failure.[16,17]

In animal studies, CoQ10 does not demonstrate genotoxicity, reproduction toxicity, or chronic toxicity.[16] In a clinical trial on patients with heart failure, CoQ10 has been dosed as 300mg/day as 3 separate doses and has shown a reduction in mortality and heart-related events.[18] Further, human studies has shown, that up to 1200 mg/day is not related to toxicity or increased numbers of side-effects, compared to 60 mg/day[19]. In Parkinson patients, 2400mg/dag in 16 months did not demonstrate serious adverse events. Also, in ALS patients, 3000mg/day in 8 months has been dosed without serious adverse events [16,19].

We aim to use a dose of 500 mg/day, dosed as 100mg x 5, for 6 weeks (against 6 weeks placebo).

3.1.2 Interactions and Safety

In the product sheet, for CoQ10 [20] a possible interaction between warfarin and CoQ10 has been described at the time of initiation of anticoagulant therapy. However, a randomized, double blinded, placebo controlled cross-over study with 24 patients has demonstrated that there is no clinical relevant interactions between warfarin and 100 mg CoQ10 daily.[21] We will however exclude participants on warfarin as our planned dosing regimen is 5 times higher. Data regarding pregnant and breast-feeding women is insufficient, as well as data on kidney disease and reduced liver function, why these groups will not be included in the study. The product contains soy oil (soy protein) and is thus contraindicated in patients with allergy to soy or peanuts.

3.1.3 Pharmacokinetics

CoQ10 is a naturally occurring lipid-soluble component of cellular metabolism and is synthesized by animals, humans and plants. It is not a vitamin, as it is endogenously synthesized.[12] CoQ10 exists in three redox states, fully oxidized (ubiquinone), partially reduced (semiquinone or ubisemiquinone), and fully reduced (ubiquinol). The pharmacokinetic profile of ubiquinol and ubiquinone is similar. Both forms are lipid-soluble and gradually absorbed from the GI tract.

Ubiquinol is the active form of CoQ10, circulating in plasma, as ubiquinone is converted to ubiquinol in the enterocytes. For ubiquinol, time to maximum concentration (T_{max}) is reached at 6h with a second peak after 12–24h. The estimated half-life ($T_{1/2}$) of ubiquinol is 48h. In single-dosing of 150mg and 300 mg, the maximum concentration (C_{max}) and $AUC_{0–48h}$ derived from the mean plasma ubiquinol concentration-time curves were 1.88 and 3.19µg/ml, and 74.61 and 91.76 µgh/ml, respectively, showing increases dose-dependently[12,22].

3.1.4 Side Effects for CoQ10

Known side effect are listed in the product resume on the website of the Danish Medicines Agency (http://produktresume.dk/)[20]

Organ class and frequency	
Nerve System Rare (>1/10.000 and <1/1000)	Headache Dizziness
Gastro-intestinal Rare (>1/10.000 and <1/1000)	Nausea, Obstipation, diarrhea, ingestion
Skin and subcutaneous tissues Very rare (<1/10.000)	Rash, itching
Psychiatric disturbances Very rare (<1/10.000)	Irritability

4 Study Objectives and Endpoints

4.1 Symptoms score

As no diagnostic test or objective analysis specific for LTC has been described, we will use a symptoms scoring system based on 2 questionnaires: the EQ-5D-5L[23] and a LTC specific questionnaire developed on the basis of data collection in the Post COVID-19 Outpatient Clinic[24] See section 7.7 and APPENDIX 1 for further details.

4.2 Objectives

4.2.1 Primary Objective

- To assess the effect of 6 weeks of CoQ10 treatment on the number and severity of self-reported symptoms in LTC patients.

4.2.2 Secondary Objectives

- To assess a potential persistent effect of CoQ10 after 6 weeks dosing and 4 weeks of follow-up on the number and severity of self-reported symptoms in LTC patients.
- To assess the safety and tolerability of the IMP

4.2.3 Explorative Objectives

- To investigate occurrence of auto-reactive antibodies at baseline compared to healthy controls (biobank samples).
- To assess CoQ10 levels in plasma before and after CoQ10 treatment

- To investigate if certain host genetic factors predict LTC
- To perform quantitative proteomics of PBMCs by high mass accuracy nanoLC-MS/MS.
- To assess cellular metabolic activities by Seahorse analyses of PBMCs samples
- To assess oxidative stress in plasma through the marker 8-isoprostane (ELISA).
- To assess a panel of cytokines in plasma through antibody based multiplexed analyses.
- To assess metabolites of kynurenic pathway in plasma by UPLC-MS/MS.

4.3 Endpoints

4.3.1 Primary Endpoint

1. Number and severity (symptoms score) of self-reported symptoms of LTC, measured by questionnaires (APPENDIX 1) before and after 6 weeks of CoQ10.

4.3.2 Secondary Endpoints

1. Duration of a potential persistent effect of CoQ10 measured as number and severity of symptoms of LTC 4 weeks after ending IMP dosing.
2. Safety evaluation, as measured by adverse events (AEs), Adverse Reactions (ARs), serious adverse events (SAEs), Serious ARs (SARs).

4.3.3 Potential Exploratory Analyses

1. Investigation of auto-reactive antibodies at baseline compared to healthy controls (biobank samples), by immunohistochemistry and enzyme-linked immunosorbent assay (ELISA) against tissue proteins.
2. Assessment of baseline levels of plasma CoQ10, measured by High Performance Liquid Chromatography (HPLC), as this parameter previously has been associated with fatigue.
3. Investigation of host genetic factors predicting LTC by tissue-type determination and single nucleotide polymorphism (SNP) analyses.
4. Differential analysis of the proteome (LC-MS/MS) and metabolic activities (Seahorse) in PBMCs from CoQ10 treated patients versus placebo.
5. Differential analysis of plasma samples from CoQ10 treated patients versus placebo, with respect to the oxidative stress marker 8-isoprostane, cytokines and metabolites of the kynurenic pathway.

5 Study Design and General Procedures

5.1 Design

This is an investigator-initiated, randomized, placebo-controlled, double-blinded, 2x2 crossover interventional trial to evaluate the effect of CoQ10 in LTC patients. Participants will be randomized 1:1 to receive CoQ10 or placebo for 6 weeks. Subsequently, a 4 weeks washout period and crossover is effectuated and patients are allocated to the opposite treatment for 6 weeks. Final follow-up period is week 16 – week 20. Two questionnaires (APPENDIX 1) are completed at baseline, after 6 weeks, 10 weeks, 16 weeks and 20 weeks. Targeted enrollment is 60 participants in each study arm (figure. 1).

5.1.1. Figure 1: Outline of study design

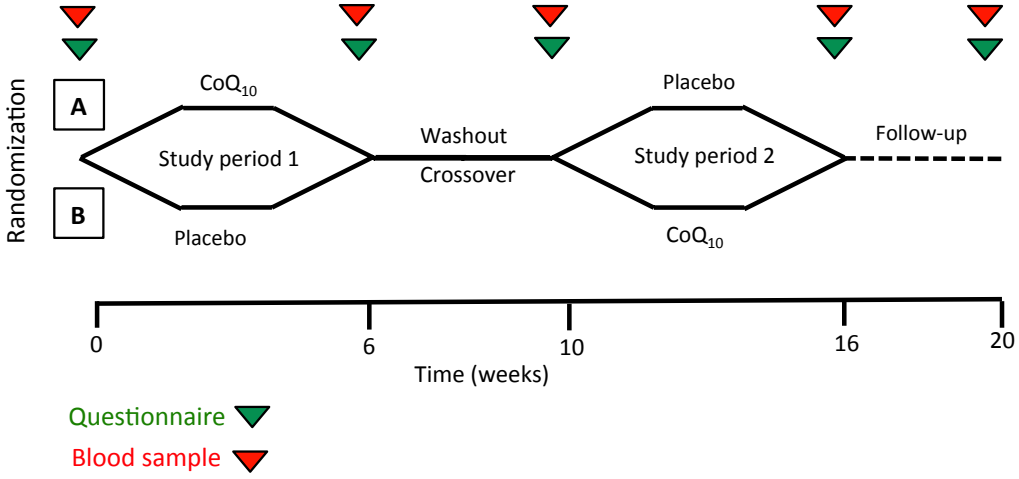

Figure 1: Illustration of study design. At baseline (week 0) participants are allocated to either 6 weeks of CoQ10 or placebo and two questionnaires (green arrows) is completed (study period 1). After 6 weeks, a 4 weeks washout period and crossover is effectuated and participants are allocated to the opposite intervention. After 16 weeks (study period 2), the questionnaires are repeated and the study is un-blinded. Blood sample (red arrows) are collected for exploratory analyses.

5.1.2 Design considerations

To minimize the risk bias from carryover effect, we have added a washout period between study period 1 and 2. As $T_{1/2}$ for CoQ10 is 48h, a 4 weeks period is considered sufficient.
It is possible that some participants will improve spontaneously over time, or experience minor improvement due to a placebo effect. The strength in the crossover design is thus, that we can use the study period 1 of arm B to evaluate the effect of time and placebo. This improvement can be used for statistical adjustments in the final analysis. A spontaneous reduction of 20 points in symptoms score is included in the power calculation to adjust for this effect when deciding targeted enrollment (se section 11.1 sample size determination). Analysis of primary outcome will take place when the last participant has completed the second dosing period (week 16) to prevent premature unblinding.

5.2 Health Facilities

This trial will be conducted at the Department of Infectious Diseases at Aarhus University Hospital (AUH) in The Long Term COVID-19 Outpatient Clinic.

5.3 Randomization

Randomization of participants will by effectuated using a central computerized system at Aarhus University Hospital that is accessible 24 hours a day, 365 days a year[25]. The aim is to include equal number of participants in both arms. Permuted block randomization in blocks of 4 and 8 will be implemented to ensure correct randomization. No pre-randomizing stratification of participants will occur. The electronic case report form (REDCap Database) will contain a concealed de-identification code, if premature unblinding is needed. The process of premature blinding is logged and monitor will have access to all logging data at the end of the study, thus able to confirm that

no inappropriate unblinding has occurred. After participant identification numbers and randomization to study arm have been assigned, no reassignment is possible.
On the site participant identification list, the site personnel will register participant identification number, randomization date, gender, last and first name, medical records identification (if applicable), and date of birth. All study personnel who directly interact with study participants are blinded to study arm designation. The personnel receiving the IMP/placebo are blinded.

5.4 Discontinuation of Study

Study subjects can withdraw from the study or the IMP in accordance with the conditions and procedures described in section 6.2 (Participant Withdrawal from Study).

Premature discontinuation of the study may occur because of a regulatory decision, change in opinion of The Ethics Committee or drug safety problems. The sponsor also has the right to temporarily suspend and/or discontinue the study due to, but not limited to, the safety of study subjects, ethical reasons, or serious problems of recruitment.

5.5 Source Data

Source data is defined as:

1. Informed consent and power of attorney
2. Information from electronic patient records (EPJ)
    - Note of each visit in the patient's file
    - Biochemical and exploratory analyses measurements
    - Concomitant medicine: List of current medical treatment
3. SAE reports

The CRF or EPJ will be the source data for:

1. Demographics: Name, date of birth, age, sex (male, female), race/ethnicity, study identification number.
2. COVID-19 medical history: regarding positive test date(s), hospitalization, outpatient visits related to acute COVID-19 illness or long term COVID-19.
3. LTC symptoms type and severity.
4. Medical history: Brief medical history, including list of medical conditions with year, treatments and sequelae.
5. Visit dates.
6. Anthropometric data: Weight, height.
7. Physical exam: Signs and symptoms noted by medical examination.
8. Pregnancy status.
9. AE, AR, SAE, and SAR reported.
10. Phone contact.
11. Extra visit.

6 Selection of Study Participants

6.1 Inclusion and exclusion criteria

6.1.1 Inclusion Criteria

1. Age above 18 years.
2. Able to give informed consent.
3. History of documented SARS-CoV-2 infection either by RT-PCR or antibody test.
4. Symptoms related to LTC, defined as being investigated, diagnosed and followed by specialized infectious diseases physicians in the Long Term COVID-19 Outpatient Clinic, Central Region of Denmark, Aarhus University Hospital. This clinic covers all LTC patients referred from general practitioners and regional hospitals in Region Midtjylland.
5. Symptoms not attributable to other co-morbidity/condition.

6.1.2.Exclusion Criteria

1. Symptoms of acute COVID-19, as defined by The Danish Health Authorities/Sundhedsstyrelsen.
2. Women who are pregnant or breastfeeding, or with a positive pregnancy test as determined by a positive urine beta-human chorionic gonadotropin test during screening
3. Hypersensitivity to the active ingredient or to any excipient of the medicinal product
4. Known allergy to soy or peanuts.
5. Individuals with reduced kidney or liver-function.
6. Patients in anticoagulant therapy with vitamin K antagonists.
7. Any condition that, in the Investigator's opinion, will prevent adequate compliance with study therapy.

6.2 Contraception and women of childbearing age

Women of child bearing potential will be asked for consent to use an acceptable method of contraception (combined estrogen and progestogen hormonal contraception (oral, intravaginal or transdermal), progesteron-only hormonal contraception (oral, injectable or implantable), intrauterine device or intrauterine hormone-releasing system) to avoid pregnancy during the first 16 weeks of study. Sexual abstinence will only be accepted in cases where this reflect the usual lifestyle. Duration of contraception must be corresponding to at least 5 $T_{1/2}$ of the IMP, i.e. 240h or 10 days after last dose in the study.

6.3 Participant Withdrawal from Study

Participant withdrawal from study is defined as any participant who does not complete the two dosing periods (i.e. week 16).

Reasons for withdrawal from the study include, but are not limited to:

- Withdrawal of informed consent (participant request).
- Protocol violation.
- Any condition, interaction, or contraindication where continued participation in the study will result in an unacceptable risk for the participant, as assessed by the Investigators or participant's physician at the Post-COVID-19 Clinic.
- Discontinuation of the study by the Sponsor.
- Lost to follow-up.

Subjects withdrawn from the study after the point of randomization will not be replaced. If possible we will obtain data on these individuals in form of questionnaires on the time of withdrawal and at end of study (study week 20)

7 Study Treatment and Specific Procedures

7.1 The Investigational Medicinal Products (IMPs)

7.1.1 IMPs

- The IMP is Coenzyme Q10, ubiquinone (CoQ10). Dosing is 500 mg/day divided as 5 doses of 100 mg.

7.1.2 Placebo

- Placebo for IMP is a soft gelatin capsule containing α-tocoferol.

7.2 Drug Supplies, Packaging and Labeling

7.2.1 CoQ10 and placebo

Capsules with CoQ10 or placebo will be delivered to study site in 2 separate boxes. Boxes with CoQ10 and placebo are identical, except for numbering with batch number and code for identification. Whether the boxes contain IMP or placebo cannot be identified from batch number. The IMP will be labeled and blinded from the supplier PharmaNord.

7.3 Crossover of Dosing Regimen

All participants will receive 6 weeks of IMP and 6 weeks of placebo in a random order according to the randomization outcome. A washout period of 4 weeks is included between treatment periods.

7.4 Drug Storage and Accountability

The Sponsor and/or Investigators at The Department of Infectious Diseases are responsible for correct storage of all IMPs after arrival at study site. The IMPs will be stored in appropriate conditions in a secure location with controlled access. The storage compartment shall be monitored regularly. Lists of received, used and remaining quantities of the IMPs will be kept. Any discrepancies must be solved. The IMP and the placebo can be stored for 5 years.

7.5 Administration

All IMPs will be ready for hand-out when the first participant is enrolled. On baseline and at crossover visit (after 6 weeks), participants receive boxes containing either IMPs or placebo to be dosed at home on a daily basis. Blinded study personnel/investigators will instruct participants in correct dosing of either IMP or placebo.

7.6 Compliance

Participants will be instructed to return empty packing material after the 6 weeks of treatment for counting in order to register compliance.

7.7 Symptom score

No diagnostic test, objective analysis or validated questionnaire specific for LTC has been published yet. Therefore, we will use a symptoms scoring system based on two questionnaires concerning quality of life and LTC specific symptoms: (1) EQ-5D-5L (5 items graded 0–4)[23] and (2) LTC specific questionnaire based on symptoms reported from a Danish cohort of LTC patients[24], (32 items, graded 0–4).
The EQ-5D-5L questionnaire is used for assessment of LTC symptoms in other studies[26], as well as in studies of ME/CSF[27]. The two questionnaires contain in total 37 items with a graduation of 0–4. Maximum number of points is thus 148, implying a high number of severe symptoms across all parameters. The questionnaire will be completed at each study visit by the participant alone. If the study participant need assistance, the investigator will be available to answer questions or assist in any other way.

7.8 Consent Procedure

Prior to initiation of study related procedures, the potential participant will be given a copy of the most recent participant information sheet and informed consent to read. Additionally, the principal investigator or a study physician who has been designated to consent will discuss the details of the study including but not limited to the purpose of the research, procedures, time commitment, required tasks, IMP, alternative treatments, benefits, risks, confidentiality etc. in a understandable (non-scientific) way, using language readily comprehensible by the participant. Any conversation between eligible study participant or study participant and study personnel will take place undisturbed in a consulting room in Long Term COVID-19 Outpatient Clinic. Subjects will be informed that participation is completely voluntary and, if they do not wish to consent, they will not be facing any consequences. The study personnel obtaining consent will assure that participation is completely voluntary. Consideration time is minimum 24 hours.

A private, confidential setting will be provided for the potential subject to read and discuss the informed consent free from coercion, influence or constraints of time. All subjects will be given a chance to ask questions and express concerns. They will be given the option to take the consent home and discuss it with family, friends, and/or health care providers. After a subject and the person conducting the consenting signs and dates the consent, the subject will be given a copy of the signed informed consent form.

By given informed consent the participant is informed, that the source data is available to the Sponsor, the regulatory authorities and Lead Principal Investigator.

8 Study Overview

8.1 Study visits

The study comprises 4 study visits in total (see table 1). Prior to formal enrollment, written informed consent is obtained from the participant. New or previously identified signs or symptoms experienced since the last visit will result in a directed physical examination and if relevant appropriate diagnostic tests.

8.1.1 Visit 1: Baseline: Day 0

Potential participants will be screened for assessment of eligibility. The following criteria will be reviewed:

- Inclusion/exclusion criteria
- If female; pregnancy test (serum/urine)
- Demographics
- Concurrent illness
- Medication history
- COVID-19 history will be obtained from EPJ
- Directed physical examination (including height and weight)
- Blood samples (40 mL).
- Questionnaires for assessment of LTC symptoms and severity.
- Instructions on dosing
- Handout of IMP/placebo

8.1.2 Visit 2: Day 42

Participants will be evaluated regarding effect of the IMP. Washout period is initiated. The following will be assessed

- Adverse events recording.
- Questionnaires.
- Directed physical examination (depending on investigators decision).
- Blood samples (40 mL).
- Returning of empty packing material from first 6 weeks dosing.

8.1.3 Visit 3: Day 70

Participants will be allocated to the opposite IMP. The following will be assessed

- Adverse events recording.
- Questionnaires.
- If female; pregnancy test (serum/urine).
- Directed Physical examination (depending on investigators decision).
- Blood samples (40 mL).
- Handout of IMP/placebo.

8.1.4 Visit 4: Day 112

The following will be assessed:

- Adverse events recording.
- Questionnaires.
- Directed Physical examination.
- Blood samples (40 mL).
- Returning of empty packing material from last 6 weeks dosing.

8.1.5 Visit 5: Follow-up – day 140

The following will be assessed:

- Adverse events recording.
- Questionnaires.
- Directed Physical examination.
- Blood samples (40 mL).

8.1.6 Table 1

Visit	**1**	**2**	**3**	**4**	**5**
Study Day Name	Baseline/ V1	V2	V3	V4	Follow-up
Week	0	6	10	16	20
Study Day	0	42 (±7 days)	70 (±7 days)	112 (±7 days)	140 (±14 days)
Informed consent	X				
COVID-19 history	X				
Demography, medical history, concurrent illness	X				
Weight/height	X				
Randomization/allocation to study arm A or B	X				
Questionnaires	X	X	X	X	X
Handout of IMPs	X		X		
Instruction on dosing	X		X		
Safety assessment		X	X	X	X
Blood samples	X	X	X	X	X
Pregnancy test (women)	X		X		
Directed physical examination	X	X	X	X	X

8.2 Blood sampling

Blood samples will be collected on all study visits. Samples will be analyzed according to the exploratory analyses outlined in section 4.2.3

8.3 Safety monitoring

Safety will be monitored by interrogation of participant's experiences of clinical signs and symptoms and direct physical examinations, as well as blood tests collected at the regular scheduled visits to the Long Term COVID-19 Outpatient Clinic. No routine safety biochemistry is collected in direct relation to the study. AEs are recorded on each study visit.

8.4 Unscheduled visits or telephone contacts

An unscheduled telephone contact or visit may be scheduled for further assessment of any AE. A medically qualified member of the study staff will assess the symptom that prompted the visit. Findings will be recorded in the eCRF as well as the electronic patient record (EPJ).

9 Efficacy Assessments

For a specification of study endpoints refer to section 4.2 (Endpoints).

9.1 Primary Endpoint

At each study visit, the investigator will interrogate participants in accordance with the questionnaires (APPENDIX 1). Change from baseline and from V3 in symptoms score will by assessed at V2 and V4 and compared to placebo.

9.2 Secondary Endpoints

1. Lasting effect of the intervention will be assessed as change in symptoms score from week 6 to week 10 for study arm A and from week 16 to week 20 for study arm B.
2. Safety and tolerability of IMP will be assessed for participants who receive at least one dose. Safety assessment will be done by recording all participant-reported AEs and SAEs.

9.3 Exploratory Endpoints and analyses

1. Prevalence of auto-reactive antibodies at baseline compared to healthy controls (biobank samples), by immunohistochemistry and ELISA against tissue proteins.
2. Assessment of baseline levels of plasma CoQ10, measured by HPLC, as this has parameter previously been associated with fatigue. Will be compared to follow-up time points.
3. Investigation of host genetic factors predicting LTC by tissue-type determination and SNP analyses.
4. Differential analysis of the proteome (LC-MS/MS) and metabolic activities (Seahorse) in PBMCs from CoQ10 treated patients versus placebo.
5. Differential analysis of plasma samples from CoQ10 treated patients versus placebo, with respect to the oxidative stress marker 8-isoprostane, cytokines and metabolites of the kynurenic pathway.

10 Safety Assessments

10.1 Adverse event reporting

10.1.1 Definitions

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding – see below), symptom, or disease temporally associated with the use of the IMP, whether or not causally linked to the investigational drug. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require intervention. In addition all cases of drug-drug interaction, pregnancy (with or without outcome), paternal exposure, lactation, lack of efficacy, overdose, drug abuse and misuse, drug maladministration or accidental exposure and dispensing errors are collected and data based even if no adverse event has been reported.

An adverse reaction (AR): When evaluating an AE the investigator assess if a causal relation to the IMP is a reasonable possibility, regardless of dosing. Thus, an AR is any AE to a medicinal product, which is noxious and unintended and deemed related to the IMP.

All adverse events will be reported according to the guidelines for Good Clinical Practice (GCP; International Conference on Harmonization 1996), and Danish ethical guidelines and law.

A serious adverse events (SAE) is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Results in persistent or significant disability/incapacity
- Constitutes a congenital anomaly/birth defect
- Requires inpatient hospitalization or prolongation of existing hospitalization, *unless hospitalization is for:*
    - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
    - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of the study drug
    - Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
    - Social reasons and respite care in the absence of any deterioration in the patient's general condition
- Is medically significant, i.e. defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above

A serious adverse reaction (SAR) is an SAE that the investigator deems related to the IMP.

Unlike routine safety assessments, SAEs/SARs are monitored continuously and have special reporting.

Pregnancies: Pregnancy, although not itself a serious adverse event, will also be reported on a Serious Adverse Event form and be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects, congenital abnormalities or maternal and/or newborn complications.

10.2 Recording of Adverse Events (AE)

At each contact with the participant, as indicated in the schedule of activities, the investigator seeks information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though they should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the

study treatment or participation is not the cause. Serious adverse events that are still on-going at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

All AE will be reported in accordance with the principles of GCP and the latest requirements of the Medicines for Human Use (Clinical Trials) Regulations.

All AEs must be scored according to the CTCAE (v5.0) and recorded on the AE form in the CRF with the following information:

1. The severity grade (mild, moderate, severe)
2. Its relationship to the IMP(s) (suspected/not suspected)
3. Its duration (start and end dates or if continuing at final exam)
4. Whether it constitutes a serious adverse event (SAE)
5. Action taken with the IMP(s)
6. Outcome

All adverse events will be graded in the following manner:

- Grade 1 (Mild): Events require minimal or no treatment and do not interfere with the participant's daily activities.
- Grade 2 (Moderate): Events result in a low level of inconvenience or concern. Moderate events may cause some interference with functioning.
- Grade 3 (Severe): Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- Grade 4 (Life-threatening): Any adverse drug experience that places the participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred (i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death).
- Grade 5 (Death)

10.3 Reporting of Serious Adverse Events (SAE)

The SAEs will be noted in the CRF. Information about all SAEs is collected and recorded on the Serious Adverse Event Report Form. The investigator must inform the sponsor and/or principal investigator, assess the relationship to study drug, complete the SAE Report Form, and send the completed, signed form email **within 24 hours** to:

Lars Østergaard, MD, PhD, DMSc
Professor/Head
Dept. of Infectious Diseases
Aarhus University Hospital,
Palle Juul-Jensens Boulevard 99, DK-8200 Aarhus N
Denmark
Ph.: +45 784 52800
e-mail: larsoest@rm.dk

The original copy of the SAE Report Form and the email must be maintained at the study site.

10.4 Suspected Unexpected Serious Adverse Reaction (SUSAR)

If the SAE is not previously documented in the package insert (new occurrence) and is considered related to the study drug, it qualifies as a SUSAR. An Aarhus University Hospital safety associate may require further information from the investigator for Health Authority reporting. Aarhus University Hospital will need to inform Pharma Nord.

The minimum necessary information to be provided at the time of the initial report includes:
An identifiable patient
An identifiable medicinal and/or pharmaceutical product
An identifiable reporter
A serious adverse event

10.4.1 Reporting of SUSARs to the Danish Medicines Authority

The study Sponsor is responsible for submission of reportable SUSARs to the Danish Medicines Authority according to regulatory requirements. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

Within 7 calendar days
Any study event that is:
Serious and
Unexpected and
Suspected and
Fatal or life-threatening

Within 15 calendar days
Any study event that is:
Serious and
Unexpected and
Suspected

The study Sponsor is also responsible for submission of SUSARs from their study to the Central Ethics Committee for their country according to local regulations.

Danish Medicines Agency
Axel Heides Gade 1
2300 Copenhagen S, Denmark
Tel.: +45 7222 7400

SUSARs will be reported by filling in and submitting the Danish Medicines Agency's e-form for reporting of SUSARs (http://laegemiddelstyrelsen.dk/en/licensing/clinical-trials/adverse-reactions/reporting-of-suspected-unexpected-serious-adverse-reactions-susars-seen-in-clinical-trials-e-form).

10.5 Submission of Development Safety Update Report (DSUR)

In addition to the expedited reporting required for SUSAR, Sponsors are required to submit a safety report to the local Health Authority and the Ethics Committee according to local regulations, once a year throughout the clinical trial or on request.

10.6 Procedure for premature unblinding

As a safety consideration for the participants, it is important that the participants study arm designation can be revealed at all times, if necessary. The electronic case report form (REDCap) database will contain a concealed de-identification code. This allows rapid unblinding of a specific study participant. The procedure is logged and monitor will at the end of the study gain access to logging data, thus making sure no inappropriate unblinding has occurred. If needed, study arm designation for a participant can be achieved by contacting study personnel (Time: 8-15, Tel. 40 45 99 62) or the doctor on duty at Department of Infectious diseases, Aarhus University Hospital (Time: 24 hours a day, Tel. 78 45 28 11)

11 Statistical Analyses

11.1 Sample Size Determination

No previous data on the effect of CoQ10 on LTC symptoms has been published. It is possible, that a placebo effect will occur, as well as some patients might experience spontaneous improvement during the study period. This improvement is estimated to be a 20 points reduction in symptoms score.[28]

Sample size determination are based on the primary endpoint; reduction in symptoms score, based on the following assumptions: Average symptoms score at baseline: 70 points, spontaneous reduction in symptoms score of 20 points during 20 weeks, with a standard deviation of ±20 points. Assuming that the interventional arm reaches a reduction in symptoms score of 30 points compared with 20 points in the placebo group after study period 1 and study period 2. Based on this assumption, 106 individuals should be enrolled in total to detect a statistically significant difference between the groups. With a 5% significance level (alpha) and 95% power targeted enrollment is 120 (60 in each arm) to accommodate for dropout.

11.2 Baseline Data

Continuous variables will be summarized using mean and confidence intervals (CI) or median and interquartile ranges (IQR), as appropriate. Numbers and percentage distribution will be quantified for categorical variables.

11.3 Primary Endpoint Analyses

The primary endpoint analysis will be performed based on the intent-to-treat population as change in LTC symptoms score from baseline to after 6 weeks of treatment compared to placebo (for arm A: study period 1; for arm B study period 2). Comparisons to baseline values will be made using two-sided student's t-test or Mann-Whitney rank sum test, depending on normality distribution. The washout period is extended to 4 weeks, thus a carryover effect is not anticipated.

11.4 Secondary Endpoint Analyses

The secondary endpoint is change in symptoms score 4 weeks after end of IMP: We aim to compare week 10 versus week 6 for study arm A and comparing week 20 versus week 16 for study arm B.
For safety analyses, the number and percentage of subjects experiencing one or more AEs will be summarized by relationship to study drug and severity. AEs will also be summarized by severity grade and by relationship to study drug according to the CTCAE v5.0.

11.5 Exploratory analyses

Will be accessed according to the type of data collected. For example paired student t-test or paired Wilcoxon test will be used to determine changes from baseline to the post-analytical treatment interruption time-point in reservoir size for each group.

P-values <0.05 will be considered statistically significant. No interim analyses will be done.

12 Access to Source Data

The Investigator will – based on The Research Ethics Committee – have direct access to all source data and documents, including patient files. The study is registered on the list of approved research projects in the Region of Central Denmark. During study monitoring, auditing, and/or inspection there will be readily access to all relevant source data and study documents for the study monitor, The Danish Medicines Authority, The Research Ethics Committee and their collaborators.

13 Study Monitoring and Quality Assurance

13.1 Sponsor and Investigator's Responsibility

It is Sponsor's responsibility to establish and maintain a quality assurance system that guarantees the quality of the study in all aspects. Sponsor can appoint qualified staff Investigators that may assist in the conduct of the study in accordance with the study protocol. All Investigators must be appointed and recorded on the study personnel list in due time before any study related procedures are carried out and must be supplied with the study protocol and all necessary information. Investigators are supervised by Sponsor or the Principal Investigator and act under their responsibility.

Sponsor will notify The Danish Medicines Agency and The Ethics Committee about the completion of the study within 90 days and within 1 year, respectively, and report the findings of the study as early as possible. If the study is prematurely terminated these agencies must be notified within 15 days and the reason for termination must be clarified.
Investigator's responsibility is to conduct the clinical trial at the study site, and if the study site consists of a team of individuals, it is the Investigators responsibility to be leader of the team.

13.2 Study Monitoring

The study is monitored by the GCP-Unit at Aalborg and Aarhus University Hospitals. The GCP-Unit and their national and international collaborators will through regular contacts, monitoring visit, telephone contacts, or written correspondence, monitor and assess the conduct of the study and contribute to high ethical, scientific, and legal standards in all aspects of the study. Sponsor, Investigators and study subjects' adherence to protocol requirements will be monitored as well as the handling of irregularities if such occur. During monitoring visits all, but not necessarily limited to, of the following issues will be discussed and assessed: informed consent, subject recruitment, follow-up, documentation, recording and reporting of AE and SAE, compliance, data quality, and data handling. Monitor will have access to all relevant data material as specified in section 12 (Access to Source Data).

13.3 Use of Case Report Forms (CRF)

Sponsor is responsible for keeping an updated and accurate electronic CRF (eCRF) intended to correctly register all observations and data related to the study. Recording in the eCRF will usually be done after every visit. Data entry into the eCRF must be done comprehensively and carefully to ensure correct data interpretation. The eCRF have a logging system, which logs; date, time, action in the eCRF and the person responsible for the action. If corrections are introduced, previous text/data will still appear in the eCRF logging system.

14 Ethical Considerations

14.1 Study Approvals

Sponsor must have prospective approval of the study protocol, informed consent documents, recruitment advertisements, and other relevant documents from The Danish Medicines Agency and The Central Denmark Region Committee on Health Research Ethics, before study initiation. The project will adhere to the Danish Data Protection Act.

14.2 Ethical Conduct of the Study

It is Sponsor and Investigators' responsibility to plan and conduct the study in accordance with the protocol, The Helsinki Declaration (1996 version), guidelines for GCP; International Conference on Harmonization 1996, and national ethical guidelines and law.

14.3 Subject Recruitment, Information and Consent

Written information and advertisements approved by The Ethics Committee and Investigator databases may be used for recruitment purposes. Eligible study participants will be recruited from the Long Term COVID-19 Outpatient Clinic at The Department of Infectious Diseases, Aarhus University Hospital. These individuals can either be invited by direct invitation in relation to a scheduled visit in the clinic or by an e-boks invitation letter, containing written information, inviting them to participate in the study. Interested subjects can contact an Investigator to receive additional information and schedule an appointment for further information, possibly attended with a companion. If the subject then wishes more time to consider participation in the study, presence of a companion, or repetition of information, a new appointment will be scheduled for this within the time plan. It is expected that the subject will either reject participation or sign the informed consent within two weeks after both written and oral information has been given.

The Investigator must ensure that each subject is fully informed about the nature and objectives of the study and possible risks associated with participation.

An Investigator will obtain written informed consent from each subject before any study-specific activity is initiated, using a consent form prospectively approved by The Ethics Committee. The study site will retain the original of each subject's consent form; a copy will be given the subject.

14.4 Harms, Risks and Inconveniences

14.1.1 IMPs

There are certain risks associated with participating in a clinical trial, as IMPs can have previously unrecognized side effects and risks. In this clinical trial the IMP, CoQ10 is regarded as a dietary supplement, which can be purchased as over-the-counter product in e.g. grocery stores. As the IMP will be dosed in a relatively high-dose, the participants will be monitored according to usual standards for clinical trials. In conclusion, the safety profile for the IMP is excellent with very few known side effects.

A possible inconvenience is the number of capsules and tablets to be taken each day.

The safety profile for the IMP is described in section 3.1.2. The label information for the IMP and placebo is included as APPENDIX 2. Safety monitoring is described in section 8.3.

14.1.2 Study Visits

To minimize the inconvenience for participants, the planned study visits have been reduced to a visit every 4–6 weeks.

14.1.3 Phlebotomy

Phlebotomy may cause some discomfort, bleeding or bruising where the needle enters the skin, and rarely, fainting or infection may occur.

14.5 Evaluation of Ethical and Scientific Aspects

The study treatment and procedures hold the risks, potential harms, and inconveniences mentioned above in section 14.4 (Harms, Risks and Inconveniences), but several benefits to the study subjects and the scientific development counterbalance these disadvantages.

The objective of this study is to test a treatment for LTC, which is currently a very poor characterized condition, with no treatment available. The IMPs bear a very low risk, while –if proven effective – the potential benefit for each individual is immediate and sensible.

The scientific value of the study is also essential, as there is currently very limited knowledge regarding LTC and both positive and negative outcomes of this study is thus of highly scientific value.

In conclusion, this study is found to be ethically justified and scientifically well balanced.

15 Data Handling and Record Keeping

15.1 Access to Personal Information

All study participants will be asked to sign a consent form at study entry allowing Sponsor and Investigators, the GCP units, and regulatory authorities to access the source data. All personnel must treat personal data as confidential.
After the informed consent is signed, information on the following aspects will be collected from the electronic patient record (EPJ) or by interrogation of the participant: (1) time for positive COVID-19 test, (2) symptoms related to acute COVID-19 illness and (3) symptoms related to LTC illness, (4) treatment and hospitalization in relation to acute COVID-19 illness, (5) data regarding age, sex, ethnicity, comorbidities, concomitant medicine, height/weight, smoking status, alcohol consumption, menstrual cycle, pregnancies, use of hormonal contraception.
The informed consent allows the investigator, sponsor, sponsors representative, study monitor, The Danish Medicines Agency, The Ethics Committee and their collaborators to have access to the study documents to monitor is all data is correct.

15.2 Handling of Personal Information

Sponsor and Investigators including designated personnel must handle and keep all study material and documents as confidential information and take steps to prevent wrongful or premature destruction of these. All personal data are protected in accordance with the Danish Data Protection Act and the EU General Data Protection Regulation (GDPR).

Blood samples and subject-specific documents will not contain information that directly identifies the subject, but will be supplied with study identification number unique for each participant. All study material will be treated in accordance with the Danish Data Protection Act.

Biological samples will be transferred as de-identifiable samples to Johan Palmfelt, and Rikke Katrine Jenstoft Olsen, both associate professors at the Department of Clinical Medicine - Research Unit for Molecular Medicine, Aarhus University. This group is specialized in conducting in-depth molecular biology analyses assessing metabolic and mitochondrial diseases, proteomics and mass-spectrometry. A data-processor agreement according to European Law will be generated prior to shipment of sample material.

Upon completion of all study related analyses all personal data will be rendered anonymous. However, according to GCP-guidelines data will remain personally identifiable for a minimum of 5 years after completion of study procedures defined as last subject's final follow-up contact. Any retained research data will be kept for a period of 20 years.

Data sharing plan: Individual de-identified participant data will be shared following the publication of the endpoints as outlined in this protocol. Data to be shared includes de-identified data points in published, peer-reviewed articles. Additional, related documents will also be available (study protocol, informed consent form, statistical analysis plan). Data will become available following publication with no planned end date. Access to the data sharing will be given to researchers who

provide a methodologically sound proposal for any type of analysis and requires IRB/Ethics committee approval (if applicable). Proposal should be addressed to larsoest@rm.dk.

15.3 Research Biobank

The biological material collected during the trial will be used for the analyses specified in this protocol. As part of a biomedical research project they also fulfill the criteria for a research biobank. Therefore, the following information is added:

- At each visit, blood draws will be performed. A total of 40 mL will be collected at each visit.
- The purpose of the research biobank is to store biological material to be used for the analyses described in this protocol (Section 9).
- Upon completion of all study-related analyses any remaining biological material will be transferred to a biobank for future research closely adhering to the Danish Protection Act. At enrollment, study subjects will be asked to give consent to storage of their remaining biological material in the biobank for future research (termination 01.01.2030).
- Regarding harms, risks and inconveniences related to collection of samples, please refer to the above section on the subject (Section 14.1)

16 Financial Issues and Insurance

Professor Lars Østergaard, Head of Department of Infectious Diseases, Aarhus University hospital, has initiated the trial and is overall responsible for the conduction. Support for funding is applied from Novo Nordisk Foundation and Research Support at Region Midtjylland. The funding will be used for running costs, laboratory analyses, reimbursement of participant's expenses, data analyses and publication/dissemination. Scientific and technical assisting personnel are hired directly into the study to carry out designated tasks. Pharma Nord (Tinglykke 4-6, 6500 Vojens) supply the CoQ10 and placebo free of charge.

There will be no wage or other benefits neither for the members of the project team or participants. None of the members of the project are associated with Pharma Nord and Pharma Nord will have no access to the person identifiable data.

Participants are under The Danish Patient Insurance that covers all study subjects as guaranteed by The Danish Act on the Right to Complain and Receive Compensation within the Health Service administered by The Patient Assurance Association.

Study subjects will be reimbursed for transport expenses relating to the study, but will otherwise not receive any financial compensation for participating in the study. Study subjects have the possibility of recovering documented lost earnings, the amount is taxable.

17 Publication of Study

17.1 Public Registration of Study

The study will be registered at https://eudract.ema.europa.eu and http://clinicaltrials.gov prior to study start upon receipt of all necessary approvals. A resume and brief outline of the study will be available according to current guidelines.

17.2 Publication of Study Results

Publication of the results of this trial will be governed by requirements for authorship declared by the International Committee of Medical Journal Editors http://www.icmje.org.

Results from the study will be published in international peer-reviewed journals, and negative, positive as well as inconclusive test results will be attempted published, the results will also be published on http://clinicaltrials.gov.

Results of the study will be made available in the EudraCT database with subsequent publication at clinicaltrialsregister.eu.

A final list of authors cannot be specified at this time, but all contributing researchers who fulfilled the Vancouver criteria for authorship will be included in the final publication.

18 Strategy for literature review

A literature search of peer-reviewed publications on the PubMed database, medRxiv and bioRxiv was conducted in November 2020. The following search terms were used alone or in combination: “Long Term COVID-19”, “Long haulers of COVID-19”, “ME/CSF”, “mitochondria”, “Coenzyme Q10”, “chronic fatigue,” “cell metabolism”, and “anti-oxidant”. Titles and abstracts were reviewed and studies that were considered relevant for the current clinical trial were included.

19 Protocol Synopsis

19.1 Title

QVID – Coenzyme Q10 for Long Term COVID-19: An investigator-initiated randomized, placebo-controlled, phase IIa trial

19.2 Background and study rationale

SARS-CoV-2 causes the disease new-coronavirus-disease-2019 (COVID-19). An increasing number of reports suggest, that persons recovered from acute COVID-19 suffer from diffuse persistent symptoms dominated by fatigue, muscle pain etc. Similar debilitating symptoms were observed in patients recovering from SARS-1 (2002–2003) and in patients with the condition Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFE). Although the etiology is unknown, evidence points towards a cellular metabolic disturbance as part of the pathogenesis. Specifically, it is possible that LTC is associated with increased cellular anaerobe metabolism resulting in decreased energy production and increased oxidative stress. Coenzyme Q10 (CoQ10) is part of the electron transport chain, responsible for the ATP synthesis in the mitochondria by oxidative phosphorylation (OXPHOS). CoQ10 is the only molecule with these attributes and is thus essential

for cellular *aerobe* metabolism. Further, it functions as an antioxidant and is constantly in an oxidation-reduction-cycle, hereby reducing the levels of free radicals. ME/CSF patients has significantly lower plasma levels of CoQ10 and severity of fatigue symptoms correlates with CoQ10 levels. In a clinical trial with ME/CSF patients, a significant improvement in fatigue was observed in patients after 8 weeks of CoQ10 supplementation compared to placebo. Given the similarities between LTC and ME/CSF it is possible, that CoQ10 supplement will improve cellular metabolism and reduce symptoms in LTC.

We aim to investigate if high-dose supplements of CoQ10 can reduce the number and severity of symptoms related to LTC.

We hypothesize that LTC-related symptoms are caused by reduced capacity for cellular OXPHOS and that high-dose supplements will significantly decrease number and severity of symptoms in LTC patients.

19.3 Objectives

Primary Objective

Evaluate the effect of high-dose CoQ10 after 6 weeks dosing on the number and severity of self-reported symptoms in LTC patients.

Secondary Objectives

- Evaluate the persistent effects of high-dose CoQ10, after 6 weeks dosing and 4 weeks of follow-up on the number and severity of self-reported symptoms in LTC patients.
- Evaluate the safety and tolerability of the IMP

Explorative Objectives

- To investigate occurrence of auto-reactive antibodies at baseline compared to healthy controls (biobank samples).
- To assess CoQ10 levels in plasma before and after CoQ10 treatment
- To investigate if host genetic factors predicts LTC.
- To investigate the proteome (LC-MS/MS) and metabolic activities (Seahorse) in PBMCs from CoQ10 treated patients versus placebo.
- To perform analysis of plasma samples from CoQ10 treated patients versus placebo, with respect to the oxidative stress marker 8-isoprostane, cytokines and metabolites of the kynurenic pathway.

19.4 Endpoints

Primary Endpoint

Number and severity of self-reported symptoms of LTC, measured by self-reporting in questionnaires (APPENDIX 1) before and after 6 weeks of CoQ10, measured by symptoms score.

Secondary Endpoints

Duration of a potential effect of CoQ10 measured as change in symptoms score 4 weeks after ending IMP: We aim to compare week 6 versus week 10 for study arm A and week 16 versus week 20 for study arm B.

Potential Exploratory Analyses

1. Investigation of auto-reactive antibodies at baseline compared to healthy controls (biobank samples), by immunohistochemistry and enzyme-linked immunosorbent assay (ELISA) against tissue proteins.
2. Assessment of baseline levels of plasma CoQ10, measured by High Performance Liquid Chromatography (HPLC), as this parameter previously has been associated with fatigue.
3. Investigation of host genetic factors predicting LTC by tissue-type determination and single nucleotide polymorphism (SNP) analyses.
4. Differential analysis of the proteome (LC-MS/MS) and metabolic activities (Seahorse) in PBMCs from CoQ10 treated patients versus placebo.
5. Differential analysis of plasma samples from CoQ10 treated patients versus placebo, with respect to the oxidative stress marker 8-isoprostane, cytokines and metabolites of the kynurenic pathway.

20 List of Abbreviations

AE	Adverse Event
AR	Adverse Reactions
AUC	Area under curve
AUH	Aarhus University Hospital
CFS	Chronic Fatigue Syndrome
COVID-19	Coronavirus Disease of 2019
C_{max}	The maximum plasma concentration
CoQ10	Coenzyme Q10
CoV-2	Coronavirus 2
CRF	Case report form
CTCAE	Common Terminology Criteria for AEs
DSUR	Development Safety Update Report
ELISA	Enzyme-linked immunosorbent assay
GCP	Good Clinical Practice
HPLC	High Performance Liquid Chromatography
IMP	Investigational Medicinal Product
LTC	Long Term COVID-19
LC	liquid chromatography
ME	Myalgic Encephalopathy
MS	Mass spectrometry
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
SAE	Serious AE
SAR	Serious AR

SARS	Severe Acute Respiratory Syndrome
SD	Standard deviation
SNP	Single nucleotide polymorphism
SUSAR	Suspected Unexpected Serious Adverse Reaction
T_{max}	Time of Cmax
$T_{1/2}$	Half-life

21 List of Figures and Tables

22 References

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APPENDIX 1

Questionnaires for assessment of LTC symptoms

EQ-5D-5L; 5 items (0–4 points; 20 points in total)

Under hver overskrift bedes du sætte kryds i DEN kasse, der bedst beskriver dit helbred I DAG.

BEVÆGELIGHED

Jeg har ingen problemer med at gå omkring ❑
Jeg har lidt problemer med at gå omkring ❑
Jeg har moderate problemer med at gå omkring ❑
Jeg har store problemer med at gå omkring ❑
Jeg kan ikke gå omkring ❑

PERSONLIG PLEJE

Jeg har ingen problemer med at vaske mig eller klæde mig på ❑
Jeg har lidt problemer med at vaske mig eller klæde mig på ❑
Jeg har moderate problemer med at vaske mig eller klæde mig på ❑
Jeg har store problemer med at vaske mig eller klæde mig på ❑
Jeg kan ikke vaske mig eller klæde mig på ❑

SÆDVANLIGE AKTIVITETER *(fx. arbejde, studie, husarbejde, familie- eller fritidsaktiviteter)*

Jeg har ingen problemer med at udføre mine sædvanlige aktiviteter ❑
Jeg har lidt problemer med at udføre mine sædvanlige aktiviteter ❑
Jeg har moderate problemer med at udføre mine sædvanlige aktiviteter ❑
Jeg har store problemer med at udføre mine sædvanlige aktiviteter ❑
Jeg kan ikke udføre mine sædvanlige aktiviteter ❑

SMERTER / UBEHAG

Jeg har ingen smerter eller ubehag ❑
Jeg har lidt smerter eller ubehag ❑
Jeg har moderate smerter eller ubehag ❑
Jeg har stærke smerter eller ubehag ❑
Jeg har ekstreme smerter eller ubehag ❑

ANGST / DEPRESSION

Jeg er ikke ængstelig eller deprimeret ❑
Jeg er lidt ængstelig eller deprimeret ❑
Jeg er moderat ængstelig eller deprimeret ❑
Jeg er meget ængstelig eller deprimeret ❑
Jeg er ekstremt ængstelig eller deprimeret ❑

LTC Specific Questionnaire – 32 items (0–4 points; 128 points in total)

	De sidste 4 uger, i hvor udtalt grad har du da oplevet:	Slet ikke (0)	En gang imellem (1)	Ofte (2)	Meget ofte (3)	Næsten hele tiden (4)
Nervesystem	Prikkende/snurrende/sovende fornemmelse i hænder/fødder	☐	☐	☐	☐	☐
	Hovedpine	☐	☐	☐	☐	☐
	Ændret/ophævet lugtesans	☐	☐	☐	☐	☐
	Ændret/ophævet smagssans	☐	☐	☐	☐	☐
Kognitivt	Koncentrationsbesvær	☐	☐	☐	☐	☐
	Hukommelsesbesvær for ting som lige er hændte	☐	☐	☐	☐	☐
	Hukommelsesbesvær for ting som skete for måneder eller år tilbage	☐	☐	☐	☐	☐
	Mental træthed	☐	☐	☐	☐	☐
Hjerte og lunger	Åndenød i hvile	☐	☐	☐	☐	☐
	Åndenød ved lavt aktivitetsniveau (fx gå på trapper)	☐	☐	☐	☐	☐
	Hoste	☐	☐	☐	☐	☐
	Hoste slim op	☐	☐	☐	☐	☐
	Brystsmerter	☐	☐	☐	☐	☐
	Hjertebanken	☐	☐	☐	☐	☐
	Tæthed i næsen	☐	☐	☐	☐	☐
	Ondt i halsen	☐	☐	☐	☐	☐
Mave, fordøjelses-system	Nedsat appetit	☐	☐	☐	☐	☐
	Kvalme	☐	☐	☐	☐	☐
	Mavesmerter	☐	☐	☐	☐	☐
	Diarré	☐	☐	☐	☐	☐
Hud	Hududslæt	☐	☐	☐	☐	☐
	Hudkløe	☐	☐	☐	☐	☐
Bevæge-apparat	Ledsmerter	☐	☐	☐	☐	☐
	Led hævelse	☐	☐	☐	☐	☐
	Muskelsmerter	☐	☐	☐	☐	☐
	Hurtig muskeludtrætning	☐	☐	☐	☐	☐
Alment, træthed, udmattelse	Fysisk udmattelse	☐	☐	☐	☐	☐
	Feber	☐	☐	☐	☐	☐
	Behov for at sove mere end du plejer om natten	☐	☐	☐	☐	☐
	Behov for at sove mere end du plejer om dagen	☐	☐	☐	☐	☐
	Søvnløshed: besvær med at falde i søvn om aftenen	☐	☐	☐	☐	☐
	Søvnløshed: besvær med at sove igennem en hel nat	☐	☐	☐	☐	☐

APPENDIX 2

Labeling of IMP and placebo

Klinisk Forsøg: QVID studiet, EudraCT 2020-005961-16
Indhold: 100 mg Coenzym Q10 (Myoquinone) eller placebo, bløde kapsler
Randomiserings-nummer: A-001
Dosering: 1 tablet 5 gange dagligt min 2 timers mellemrum
(bør indtages i forbindelse med måltid)
Opbevares ved max 25°
Opbevares utilgængeligt for børn
Kun til anvendelse i klinisk afprøvning
Pharma Nord projektnummer P-11261
Sponsor Lars Østergaard, Infektionssygdomme, AUH, telefon: +45 78 45 28 00.
Investigator: Kristoffer Skaalum Hansen, Infektionssygdomme, AUH
Batchnr.:_____ Anv. Inden:_____