Czech Anaesthesia Clinical Trials and Audits Network

CLINICAL TRIAL PROTOCOL

AZIQUINE-ICU

Azithromycin added to Hydroxychloroquine in Patients Admitted to Intensive Care due to Coronavirus Disease 2019 (COVID-19)– Randomised Controlled Trial

[Azitromycin přidaný k hydrochlorochinu v léčbě pacientů přijatých do intenzívní péče s infekcí COVID-19 - randomizovaná kontrolovaná studie]

Phase III clinical trial

Protocol Number AZIQUINE-ICU 25032020 Version 1.5

Original protocol date 25th March 2020 Version 1.4

Eudra CT number: 2020-001456-18

Clinical Trials Registration No: NCT04339816

Sponsor: Donatio Intensivistam Endowment Fund

www.donatio-intensivistam.cz

VAT No 0907206 Registered under No N1795 by Municipal Court in Prague on 2nd April 2020

Principal investigator: Dr František Duška, PhD, AFICM, EDIC

Charles University, 3rd Faculty of Medicine and FNKV University Hospital, Prague, Czech Republic, Dept. of Anaesthesia and Intensive Care

Address: Srobarova 50, 10034 Prague, Czech Republic

Phone: +420267162451 Emergency 24/7 contact number: +420608405541

Email: frantisek.duska@lf3.cuni.cz

SIGNATURE PAGE

Name and address of the contracting authority:

Donatio Intensivistam Endowment Fund

Address: Hornoměcholupská 40c/1640, 102 00 Prague 15, Czech Republic

www.donatio-intensivistam.cz

VAT No 0907206 Registered under No N1795 by Municipal Court in Prague on 2nd April 2020

Name and title of the person authorized to sign the Protocol and the Amendments to the Protocol on behalf of the contracting authority.

Dr Frantisek Duska, Founder, Chairman, Telephone +420 608405541

Signature: Date:

Principle investigation declaration:

I hereby declare that this clinical trial will be conducted in accordance with the principles of Good Clinical Practice and Czech law

[Prohlašuji/Souhlasím s tím, že klinické hodnocení budu provádět dle Protokolu, v souladu s GCP a platnou českou legislativou.]

Dr Frantisek Duska

Signature: Date:

LIST OF ABBREVIATIONS

ICU	Intensive care unit
eCRF	Electronic Care Report Form
FiO_2	Fraction of oxygen in inspired air
F-up	Follow up
LFT	Liver function tests
IL-6	Interleukin 6
MV	Mechanical ventilation
PEEP	Positive end-expiratory pressure
PIP	Peak inspiratory pressure
pCO_2	Partial pressure of carbon dioxide in arterial blood
pO_2	Partial pressure of oxygen in arterial blood
Pplat	Plateau pressure
rtPCR	Real-time polymerase chain reaction
REB	Research Ethics Board [Etická komise]
SOFA	Sequential Organ Failure Assessment.

CONTENT

ABSTRACT:

Trial design: Prospective, multi-centre, randomised, double blind trial

Methods: Participants: Adult (>18 years) within 24 hours of admission to intensive care unit with proven or suspected COVID-19 infection, whether or not mechanically ventilated. Exclusion criteria: symptoms of febrile disease for ≥1 week, treatment limitations in place or moribund patients, allergy or intolerance of any study treatment, incl. long QT syndromes, participation in another outcome-based interventional trial within last 30 days, patients taking Hydroxychloroquine for other indication than COVID-19, pregnancy.

Interventions: Patients will be randomised in 1:1:1 ratio to receive Hydroxychloroquine 800mg orally in two doses followed by 400mg daily in two doses and Azithromycin 500 mg orally in one dose followed by 250 mg in one dose for a total of 5 days (HC-A group) or Hydroxychloroquine+ placebo (HC group) or placebo + placebo (C-group) in addition to best standard of care, which may evolve during the trial period but will not differ between groups.

Objective: To test the hypothesis that early administration of combination therapy slows disease progression and improves mechanical-ventilation free survival.

Outcomes:

Primary outcome: Composite percentage of patients alive and not on end-of-life pathway who are free of mechanical ventilation at day 14.

Secondary outcomes: Composite percentage of patients alive and not on end-of-life pathway who are free of mechanical ventilation at day 14 in the subgroup of patients without the need of mechanical ventilation at baseline; ICU-LOS; D28 and D 90 mortality (in hospital)

Tertiary (exploratory) outcomes: Viral load at D7 of study enrolment (No of viral RNA copies/ml of blood); proportion of patients alive and rtPCR negative from nasal swab at D14; Difference of FiO_2 requirement and respiratory system compliance between day 0 and 7.

Randomization: In 1:1:1 ratio and stratified according to study centre and patients age (cut-off 70 years)

Blinding (masking): Patients, treating clinicians, outcome assessors and data analyst will be blinded to study treatment allocation. Unblinded study pharmacist or research nurse will prepare investigational products.

Keywords: novel coronavirus; covid-19; SARS-CoV-2; Azithromycin; Hydroxychloroquine**;** respiratory failure

Trial registration: Eudra CT Number 2020-001456-18; Registration No (www.clinicltrials.gov): NCT04339816

LAY SUMMARY (IN CZECH):

V současné době probíhá celosvětová pandemie koronavirem COVID-19. Většina onemocnění má lehký průběh, ale menšina nemocných vyžaduje hospitalizaci a několik procent nakažených umírá. Nejčastější příčinou hospitalizace je ztížené dýchání v důsledku postižení plic virózou. Příčinnou léčbu této infekce zatím neznáme, a proto se provádí léčba podpůrná – spočívající většinou v podpoře dýchání podáním kyslíku nebo umělou plicní ventilací a v indikovaných případech podáním antibiotik jako prevence nasedlé infekce bakteriální. Na buněčných kulturách a prvních datech na pacientech (v kazuistikách a pozorovacích studiích) se zdá, že by dělení viru mohlo být zpomaleno lékem hydrochlorochin (přípravek Plaquenil, který se používá v léčbě malárie nebo některých autoimunitních nemocí, jako např. revmatoidní artitida) nebo jeho kombinací s dlouho užívaným antibiotikem azitromycinem (Azitrox, Zitromax). V této studii budou pacienti, kteří byli z důvodu těžkého průběhu COVID-19 infekce přijati do intenzívní péče, náhodně vybráni v poměru 1:1:1 do 3 skupin. U všech pacientů bude probíhat standardní podpůrná léčba, jak je popsána výše. Navíc jedna skupina bude dostávat po 5 dní kombinaci hydrochlorochinu a azitromycinu, druhá hydrochlorochinu a placeba a třetí skupina bude léčena placebem, tj. přípravkem bez účinné látky. Hlavním vyhodnocovaným parametrem je procento pacientů, kteří jsou po dvou týdnech naživu a bez potřeby umělé plicní ventilace. Dále budou sledovány nežádoucí účinky, délka pobytu na jednotce intenzívní péče a vylučování viru po dvou týdnech od zařazení do studie a přežití pacientů po 28 a 90 dnech.

INTRODUCTION

BASIC INFORMATION

Names and brief description of the investigational drugs:

Hydroxychloroquine sulphate: Hydroxychloroquine, sold under the brand name Plaquenil among others, is a medication used to prevent and treat malaria in areas where malaria remains sensitive to chloroquine. Other uses include treatment of rheumatoid arthritis, lupus, and porphyria cutanea tarda. It is taken by mouth. Hydroxychloroquine is in the antimalarial and 4-aminoquinoline families of medication. It was approved for medical use in the United States in 1955 and it is on the World Health Organization's List of Essential Medicines, the safest and the most effective medicines needed in a health system.

Azithromycin is a macrolide antibiotic used for the treatment of a number of bacterial infections and malaria. It can be taken by mouth or intravenously with doses once per day. It works by decreasing the production of protein, thereby stopping bacterial growth. Azithromycin was discovered in 1980 and approved for medical use in 1988. It is on the World Health Organization's List of Essential Medicines, the safest and the most effective medicines needed in a health system. The World Health Organization classifies it as critically important for human medicine. It is available as a generic medication and is sold under many trade names worldwide.

BACKGROUND AND RATIONALE

In early 2020 novel Coronavirus disease (COVID-19) begun to spread from Asia to Europe and beyond forcing WHO to declare global pandemic. Infected patients shed the virus for a median of 20 days [1]. Up to 10% of COVID-19 infected patients develop a severe form of disease requiring intensive care admission and some of them dies. SARS-Cov2 is encapsulated positive strand RNA virus that uses ACE-2 of type 2 pneumocytes as binding sites. It has been hypothesised (Gattinoni 2020 ICM editorial) that initial hypoxemia caused by loss of primary injury to pulmonary vasculature leads to hyperventilation and patients self-inflicted lung injury predominantly in lung areas of increase stress and strain. In turn, later during the course of the disease, ARDS develops with a typical restrictive pattern of a stiff, wet and recruitable lung. At presents there is no evidence-based causative treatment of SARS-CoV-2 and there is a burning need of randomised-controlled trials to find effective therapeutic strategies intervention.

Chloroquine has been used for malaria treatment and chemoprophylaxis, and hydroxychloroquine is used for treatment of rheumatoid arthritis, systemic lupus erythematosus and porphyrias. Both drugs have in-vitro activity against SARS-CoV, SARS-CoV-2, and other coronaviruses, with hydroxychloroquine having relatively higher potency against SARS-CoV-2 known to be susceptible in vitro to exposure to

Hydroxychloroquine [2–4]. At the moment clinical trials are ongoing to test clinical efficacy in pre- and post-exposure prophylaxis in SARS-CoV-2. In one highly cited French non-randomised observational study by Gautret et. al., a significant reduction of virus carriage has been observed in patients co-incidentally treated by Azithromycin in addition to Hydroxychloroquine [5] as a part of initial empirical therapy of community-acquired pneumonia. Azithromycin is a macrolide antibiotic, which binds to the 50S subunit of the bacterial ribosome, thus inhibiting translation of mRNA. The positive-sense RNA viruses and indeed all genes defined as positive-sense can be directly accessed by host ribosomes to immediately form proteins and the effects of Azithromycin on this process are not known. No data are available at present on the clinical efficacy of Hydroxychloroquine alone or in combination with Azithromycin and it is likely that any treatment affecting virus replication would only be effective if administered early, before overt ARDS develops.

In the light of this, we designed a trial in which we test a hypothesis, that early administration of hydroxychloroquine alone or in combination with azithromycin can prevent respiratory deterioration in patients admitted to intensive care due to rapidly progressive COVID-19 infection.

OBJECTIVES AND OUTCOMES

OBJECTIVES

Primary study objective is to test the hypothesis that early administration of combination therapy slows disease progression and improves mechanical-ventilation free survival.

Secondary (exploratory) study objectives is to investigate, whether the intervention decreases viral load and influences oxygenation and mechanical feature of the lung.

OUTCOMES

Primary outcome:

- Composite percentage of patients alive and not on end-of-life pathway who are free of mechanical ventilation at day 14 (see detailed explanation below)

Secondary outcomes:

- Composite percentage of patients alive and not on end-of-life pathway who are free of mechanical ventilation at day 14 in the subgroup of patients without the need of mechanical ventilation at baseline.
- ICU-LOS
- D28 and D 90 mortality

Tertiary (exploratory) outcomes:

- Viral load at D7 (No of viral RNA copies/ml of blood)
- Proportion of patients alive and with negative rtPCR nasal swab at D14
- Difference of FiO_2 ratio between day 0 and 7.
- Difference of respiratory system compliance between day 0 and 7.

Comment to primary outcome: Mechanical ventilation is defined as the need at any time during the last 24 hours of positive pressure ventilation, shall it be ventilation via endotracheal tube, tracheostomy or non-invasive ventilation delivered by a tightly fitting mask. On the contrary, high-flow nasal oxygen support, positive airway pressure via a helmet or uninterrupted spontaneous ventilation via tracheostomy (incl. with the use of Ayre T-piece with or without PEEP ventil) is NOT considered mechanical ventilation. End-of-life care is defined as compassionate care in patients nearing end-of life. Isolated do-not-resuscitate or do-not reintubate decisions or other ceilings of treatments are NOT considered end-of-life care, provided that decision is made to continue current level of treatment and actively support patient's improvements.

TRIAL DESIGN

Trial design: Prospective, multi-centre, randomised, double blind trial

Overview of study procedures:

Eligible patients will be randomised in 1:1:1 ratio to receive Hydroxychloroquine 800mg orally in two doses followed by 400mg daily in two doses and Azithromycin 500 mg orally in one dose followed by 250 mg in one dose for a total of 5 days (HC-A group) or Hydroxychloroquine+ placebo (HC group) or placebo + placebo (C-group) in addition to best standard of care, which may evolve during the trial period but will not differ between groups. Randomisation will be stratified according to study centre and subject's age (<70 or ≥70 years). The study medication will be prepared in each centre by a dedicated unblinded pharmacist/nurse-assistant and handed over to the treatment team in an opaque syringe or mug, respectively, depending on whether the study medication is to be administered by mouth or into a nasogastric tube. Both study subjects and the treatment team will be blinded to treatment allocation. Throughout the treatment, subjects' vital functions will be monitored continuously in addition to at least twice weekly 12 lead ECG, and laboratory parameters of liver and renal functions. All adverse events will be collected daily and serious adverse events reported as per current guidelines. The patient will have study visits daily as long as he/she stays on ICU or until day 14, whichever occurs earlier. Primary outcome will be recorded at day 14 after enrolment, in hospitalised patients during study visit, in discharged patients over the phone. At discharge from ICU (which may occur before or after day 14) as summary complications and concomitant treatments will be recorded in eCRF. Study subjects will be followed up at days 28 and 90.

DEFINITION OF STUDY POPULATION

INCLUSION CRITERIA

- Adult (>18 years)
- Within 24 hours of admission to intensive care unit[1]
- Proven or suspected COVID-19 infection[2]

EXCLUSION CRITERIA

- Symptoms of febrile disease for ≥1 week at enrolment
- Pregnancy or inability/unwillingness to perform pregnancy test at and 28 days after enrolment
- Treatment limitations in place or moribund patients
- Allergy, intolerance or contraindication to any study drug, such as long QT syndromes, myasthenia gravis, pre-existing maculopathy or retinopathy, allergies or known deficiency of glucose-6-phosphate dehydrogenase
- Known HIV positivity
- Significant liver disease such as cirrhosis Child-Pugh C or active hepatitis B or C
- Known stage IV chronic kidney disease, on dialysis at enrolment or imminent need of it
- Participation in another outcome-based interventional trial within last 30 days
- Patients taking Hydroxychloroquine for other indication than to treat COVID-19.

[1] For the purpose of this study, intensive care unit is defined as a facility that allow continuous monitoring of vital functions and oxygen administration. In other cultural context this could include also high dependency unit and intermediary care units or level 2 units.

[2] It is expected that most patients will have rtPCR test known within 24 hours of admission to hospital. Nonetheless, if this is not the case (eg. due to overloaded lab facility, lack of supplies) it is possible to randomise a patient based on a strong clinical suspicion of SARS-Cov-2 infection. In case COVID-19 is not confirmed in retrospect, experimental therapy is withdrawn and the study subject is withdrawn from "per protocol" analysis of the primary and secondary outcomes, but remains in "intention-to-treat" cohort for the analysis of safety.

CRITERIA FOR WITHDRAWAL FROM THE STUDY

In general study subject will be withdrawn from the study if any of the following criteria are fulfilled:

- Consent withdrawal by study subject
- In case – in the opinion of the investigator – the study procedures are considered unsafe for the study subject or the risks outweigh the potential benefits

Specifically, for the study subjects enrolled into the study as COVID-19 suspected but without the definite result of rt-PCR testing:

- If COVID-19 is negative and believed to be true negative[3], subjects will be immediately withdrawn from the study and administration study medication will immediately be stopped[4].

[3] In case there is a strong suspicion of COVID-19 despite negative test or if there is a doubt about the validity of the test (e.g. questionable sampling technique) it is possible and recommended to repeat the test and only withdraw the subject from the study after the confirmatory test is negative.

[4] Yet, data on adverse events will still be collected and analysed, in accordance with “intention-to-treat” mode of safety analysis.

TREATMENT

STANDARD OF CARE

All patients will receive best supportive care that will be monitored, but not protocolised. It is recognised that in the standard of care may substantially differ among study centres and that is why randomisation is stratified. There is no study-specific restriction apart from open-label use of hydroxychloroquine and macrolide antibiotics. All drugs known to prolong QTc must be used with caution. The standard of care also may change in time during the course of the study, for example, if a new evidence emerges and changes the state-of-the art treatment recommendations. Any such event will trigger emergency steering committee meeting, and decision will be taken about further action. REB and regulatory authorities will be notified immediately about the decision taken (see below).

Emphasis will be put on adherence to state-of-the-art recommendations of Czech Society of Intensive Care Medicine (ČSIM, www.csim.org) that are regularly updated and issued in accordance with recommendation of other societies.

STUDY MEDICATION

All study medication is provided as a kind gift of Zentiva, ltd. Unblinded study pharmacist will prepare into 40 mls of sterile water study medication, according to patient's allocation into the treatment arm:

- Hydroxychloroquine sulphate 200mg
- Azithromycin dihydrate 500mg
- Sterile water as placebo

For possible drug-drug interactions and details of possible adverse effects see enclosed Summary of Product Characteristics for both IMPs (investigational medicinal products), which are enclosed as Appendix I to this Protocol.

Masking. The study drugs will be administered in covered Jeannete syringe into nasogastric tube (for patients unable to swallowed) or drunk from a black mug by patients who are able to. In both cases at least 50 ml of water will be used for flush.

Treatment group allocation is as follows:

STUDY GROUP

HC-A (INTERVENTION)	Azithromycin	Hydroxychloroquine
HC (ACTIVE COMPARATOR)	Sterile water	Hydroxychloroquine
C (PLACEBO)	Sterile water	Sterile water

Table 1: Treatment allocation in 3 study arms.

Each study subject will be given:

- Day 1: Patients receive two doses 400 mg of Hydroxychloroquine or placebo in 12 hours interval and 500 mg of Azithromycin or placebo once in 24 hours (with the first dose of hydroxychloroquine or placebo)
- Days 2-5: Patients receive two doses 200 mg of Hydroxychloroquine or placebo in 12 hours interval 250 mg of Azithromycin or placebo once in 24 hours (with the first dose of hydroxychloroquine or placebo)

Adjusting IP administration to patient' s swallowing capability and GI function:

During the study, we expect following patients may be enrolled. Those conscious and able to swallow will be given study medication in black mug to swallow. Patients unable to swallow with a nasogastric tube in place will be given the IP via the NG tube reconstituted in sterile water and flushed as per local NG medication guideline. Administration of the IP is temporarily omitted in patients who are unable to swallow but without NG access or do not tolerate any enteral intake (such as patients in profound shock). As soon as the condition preventing IP administration is eliminated, resumption of study medication follows the guidance for day 1.

For per protocol concomitant medication policy, see Treatment section below.

Emergency unblinding procedure

In case the Investigator suspects that an adverse event had occurred that represents a threat to patient's safety, it is possible to unblind study treatment allocation. Unblinded pharmacist will insert a card with patient's treatment allocation into an opaque sealed envelope labeled "Emergency unblinding only, do

not open" into patients medical chart. The Principal Investigator must be notified about the use of emergency unblinding procedure within 24 hours.

PROTOCOL IMPLEMENTATION AND STUDY PROCEDURES

Prior to initiation of each centre, the central research coordinator will train the local dedicated study personnel in the use of e-CRF and other study procedures. The medication will be delivered via a central pharmacy in sealed numbered boxes and stored in ICUs. Adhering to Good Clinical Practice rules and guidelines is of upmost importance despite all the challenges during current pandemic situation. Nonetheless, the eCRF has been designed to balance the safe conduct of the clinical trial and feasibility of timely data entry during staff shortages and overload. In order to do so, all data that is not essential for safety can be input in retrospect. Data on interventions that we know/think influence survival will be monitored and described. This include concomitant antiviral antimicrobial diagnosis. We will also collect data on how the respiratory specimen for rtPCR diagnostic was collected (smear, endotracheal aspirate, bronchoalveolar lavage).

	STUDY PERIOD											
	Enrolment	Allocation	Post-allocation								F-up	
TIMEPOINT**	0-24h	0	$D_{1,2}$	D_3	D_{4-5}	D_6	D_7	D_{8-13}	D_{14}	D/C	D_{28}	D_{90}
ENROLMENT Eligibility screen	X**											
Informed consent	X									(X)	(X)	(X)
rtPCR COVID-19	X								X			
Randomisation (stratified)		X										
INTERVENTION (IP administration)												
AZI/placebo			⟷	⟷	⟷							
HCQ/placebo			⟷	⟷	⟷							
ASSESSMENTS												
Adverse events		X	X	X	X	X	X	X	X	X	X**	
ECG	X			X			X					
MV status and parameters	X		X	X	X	X	X	X	X*	X		
LFT, crea	X			X			X					
SOFA,	X						X		X			
Cointerventions + cultures + co-medicat										X		
Vital status			X	X	X	X	X	X	X*	X	X	X
Store plasma for viral load and inflammatory status	X			X			X					

Table 2. Overview of study procedures. Follow-up can be performed over the phone in outpatients. Note: Co-med = concomitant medication, D/C=discharge from ICU, F-up = follow up, LFT = liver function tests; MV=

mechanical ventilation, SOFA = sequential organ failure assessment.)* Primary outcome = being alive and off MV at D14.)** This includes pregnancy test if applicable.

Study procedures are summarised in Table 2. It is noted that most data relevant for the study will be collected as part of routine care of patients in intensive care unit.

SCREENING

All patients admitted to ICU with suspected or confirmed COVID-19 will be screened for eligibility and their basic demographic characteristics entered into the electronic screening log. All eligible patients will be approached and asked to participate in the trial.

INFORMED CONSENT PROCEDURE

Patients with decision-making capacity will be asked to provide written prospective informed consent (See Appendix II) to this protocol. It is expected that a significant proportion of screened patients will lack the capacity to provide informed consent due to altered consciousness, respiratory distress or sedation to facilitate mechanical ventilation. In this situation, the deferred consent policy will be applied as per local law: independent physician will confirm in writing that the patient lacks capacity and fulfils criteria. Then, patient himself/herself are approached to provide consent as soon as they regain capacity. They are given options to continue in the study, to withdraw with permission to use the data collected up to the point or to withdraw from the study and request deleting all data collected. Patient next of kin plays no formal role as surrogate decision maker as per Czech legislation. Nonetheless, the family will be informed when practical about their relative's enrolment into the trial and the family will be offered an information leaflet explaining the nature of the study. All serious adverse event that are suspected from being related to study interventions will be reposted to Research Ethics Board and regulatory authorities as per local legislation.

ELIGIBILITY

Prior to randomisation, all consenting patients will be checked against inclusion and exclusion criteria. These include a pregnancy test in all women with child bearing potential.

RANDOMISATION

Will be performed in blocks of 6 and stratified for study centre and age (above or at/below 70 years). Electronic Case Record Form (eCRF) will perform randomisation using random sequence script in software R and generates medication code. Rationale: Patient's age is single most powerful predictor of outcome and stratifying randomisation and stratification decreases probability of treatment groups being of different age at

baseline by chance. In analogy, study cente.rs may vary in the use of non-protocolised treatments, which could bias the results.

ENROLMENT

At baseline, following data will be collected for each patient (as guided by the respective open text or scroll-down field in eCRF):

- Onset & Admission: Onset date of first/earliest symptom, admission date and time to hospital, maximal RESPIRATORY SUPPORT within last 24 hours (PEEP [mbar], PIP [mbar], Pplat [mbar], Respiratory rate [bpm], FiO2 [21-100%], Respiratory system compliance [mL/mbar], Airway resistance [mbar/L/s]), PaO2 [kPa], PaCO2 [kPa], pH, Lactate [mmol/L]
- Charleson Comorbidity Index (as per https://www.mdcalc.com/charlson-comorbidity-index-cci)
- ECOG functional status prior to admission (scroll-down list):

  0 Fully active, able to carry on all pre-disease performance without restriction

  1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work

  2 Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours

  3 Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours

  4 Completely disabled; cannot carry on any selfcare; totally confined to bed or chair

  5 Dead

- SOFA Score which includes components of
  - Respiratory system - PaO2/FiO2 (mmHg)
  - Nervous system - Glasgow coma scale
  - Cardiovascular system - Mean arterial pressure OR administration of vasopressors required
  - Liver - Bilirubin (mg/dl) [µmol/L]
  - Coagulation - Platelets×109/L
  - Renal function: Kidneys - Creatinine (mg/dl) [µmol/L] (or urine output)
- COVID-19 status (proven/suspected as per rtPCR test results) and concomitant significant microbiology findings
- 12 lead ECG will be obtained and QTc interval measured and recorded.

- BASELINE PLASMA SAMPLE 2x1 ml: Taken and put on –20 C: for later analysis of IL-6 ferritin and other inflammatory markers as well as quantification of viral load (No of viral RNA copies per ml)

PROCEDURES ON DAY 1 UNTIL DAY 14 OR PATIENT'S DISCHARGE FROM ICU

Patient's vital status, parameters of respiratory support as above plus whether the patient has been prone-ventilated and for how long, administration of vasopressors and vital status will be checked and recorded.

In addition, 12 lead ECG, blood creatinine and liver function tests will be checked on day 3 after enrolment. On day 3 and 7. On day 7, SOFA score will be recorded again, and follow-up plasma sample taken and frozen for the same analyses as above.

PROCEDURES ON DAY 14: ASSESMENT OF PRIMARY OUTCOME

In patients still on ICU, day 14 is the last day of routine daily visits. In addition to it, the investigator decides whether the patient is alive, not on end-of-life pathway and free of mechanical ventilation (primary outcome) and patients will be tested by rtPCR from nasopharyngeal smear.

In patients who are not on ICU, but still in hospital, assessment of primary outcome is performed by physical visit to study subjects and patients will be tested by rtPCR from nasopharyngeal smear.

In patients who are discharged or transferred to other facility, primary outcome is assessed over the phone. At least 3 attempts to contact in 2 separate days are made. If still unsuccessful it is assumed that have the same primary outcome status as that had had at discharged from hospital (i.e. patients who were discharged alive and not on end-of-life pathways are still alive etc).

DAY OF PATIENT'S DISCHARGE FROM ICU

At discharge from ICU, data on following concomitant treatments throughout ICU stay will be collected:

- antibiotics (drug, no of days of administration) incl. clinically significant microbiology findings
- antivirals (drug, no of days of administration)
- immune suppressive drugs such as steroids or IL-6 antagonists (drug, no of days of administration)
- anticoagulation treatment (drug, **dose**, no of days of administration)

Also, a review of complications of the underlying disease and organ support will be noted, including summary of days on the ventilator as guided by the respective part of eCRF.

DAY 28 FOLLOW UP

Day 28 follow up will be performed as physical visit in hospitalised patients or a telephone interview. It includes vital status, mechanical ventilation status, whether the patient is still in ICU and in hospital. In hospitalised patients of child-bearing potential, pregnancy test is repeated. In discharged patients, ICU and hospital length of stay is recorded. It is recommended that patient's self-reported data are checked against hospital notes and/or clinical information system.

DAY 90 FOLLOW UP

Day 90 follow up will be performed as physical visit in hospitalised patients or a telephone interview. It includes vital status, mechanical ventilation status, whether the patient is still in ICU and in hospital. In discharged patients, ICU and hospital length of stay is recorded. It is recommended that patient's self-reported data are checked against hospital notes and/or clinical information system.

In addition actual ECOG performance status will be noted again.

0 Fully active, able to carry on all pre-disease performance without restriction

1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work

2 Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours

3 Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours

4 Completely disabled; cannot carry on any selfcare; totally confined to bed or chair

5 Dead

DATA PROCESSING AND STATISTICS

SAMPLE SIZE CALCULATION

Based on data from Wuhan [6] and Washington [7], where 67% of patients were dead in 2 weeks and half of the survivors needed protracted mechanical ventilation, we assume the incidence of the primary outcome in the control group (i.e. being alive and off the ventilator in 2 weeks) to be 25%. The study gives us 80% chance to detect the increase of the primary outcome to 50% in one or both interventional groups at $p<0.017$[5] with 74 patients in each arm. In order to compensate for drop-out and low of follow up we plan to enrol 240 subjects into the study. No replacement of subjects who dropped out from the study is planned.

STATISTICAL METHODS AND INTERIM ANALYSIS

Proportion of patients alive and off mechanical ventilation (primary outcome) between intervention and control groups will be calculated using chi square test. P-value is adjusted for multiple comparisons to p=0.017 as there are 3 comparisons between 3 study arms. Survival and ICU/length of stay will be compared using Kaplan-Maier's curves and exploratory analyses my multi-level regression using statistical packages in software in R. We plan to analyse the primary outcome separately in patients who require mechanical ventilation at baseline from those who don't and we in patients above or below 70 years of age as *a priori* subgroup analyses.

We plan to perform an interim analysis after the primary outcome is known for the 120th subject. As mentioned above, primary and secondary outcomes will only be calculated in patients with confirmed COVID-19 infection who received at least one dose of IP. Adverse events will be calculated in all randomised patients (intention-to-treat analysis).

[5]This P-value is achieved as $p<0.05$ adjusted for multiple comparisons as there are 2 control groups in this study.

SOURCE DATA PROTECTION, INTEGRITY AND QUALITY ASSURANCE

All data will be stored in password-protected, custom-programmed Oracle-based database linked to eCRF and stored and backed-up in secured servers located within hospital premises. Primary imputation of data into the eCRF will be performed by investigators or a dedicated study personnel under the supervision of investigators. Investigators vouch for source data quality and integrity.

TRIAL MONITORING

The eCRF is designed to generate queries and reports on missing data or unusual data entries, which will be fed back to study site investigators in regular intervals. Study monitoring will be performed by a dedicated study monitor

Mgr. Veronika Měrková

Email: veronika.merkova88@gmail.com

Phone +420 777 556 113

ETHICAL CONSIDERATIONS

The trial design is in accordance with Declaration of Helsinki and the protocol, case report form and informed consent formularies were reviewed and approved by FNKV University Hospital Research Ethics Board ("Ethical Committee") on 1st April 2020 (decision number KH 14-00-2020). This review included details of enrolment of vulnerable subjects, in particular those who are unable to consent for themselves, because they lack capacity due to severe distress or sedation and mechanical ventilation. The procedure of deferred consent, described in detail above, was chosen for the purpose of this trial.

BENEFITS AND RISKS ANALYSIS.

This trial investigates two drugs that are being used off label to treat patients with severe COVID-19 with a balance of potential benefits and harms. Potential benefit include good biological rationale and observational data [5] to believe that one or both drugs can prevent progression of a disease which led to death in 67% of patients in similar condition to those we plan to randomise [7]. Possible harms include QT prolongation and resulting life-threatening arrhythmias or retinopathy, which may result in blindness. We believe that clinical equipoise regarding risk/benefit to individual patient justifies well the conduct of this RCT.

STOPPING RULES

All SUSARs will be reported to both REB and to the steering committee, who may decide to stop the trial for safety concerns. Apart from safety, other reasons for stopping the trial are:

1. Emergence of new data (e.g. publication of the results of a big RCT) that may lead continuation of the trial unethical. This may be due to safety concerns of placebo group (in case strong clinical benefit is proven by other trial) or any of the interventional groups (e.g. if harm is reported by other trials). This rule also applies for emergence of a new treatment.
2. After interim analysis: The steering committee will review primary outcomes and the summary of adverse events in all 3 groups (labelled as A, B, C) whilst still blinded to treatment allocation. The treatment can be stopped if the following criteria are fulfilled:
    a. There is a significant difference in the primary outcome at $p<0.017$ in between the groups.
    b. Futility: The futility criterion is not binding for the steering committee. Based on available data the study statistician calculates the probability of being able to prove the null hypothesis with achieving the target number of subjects and the probability of type II error made by stopping the trial prematurely.
    c. Safety: In case the number of adverse events in one or more treatment groups is find uneven or unacceptably high.

Details of pharmacovigilance are described in a dedicated section below.

REPLICATION OF KEY ASPECTS OF TRIAL METHODS AND CONDUCT AND DISSEMINATION OF RESULTS

The trial is designed to be fully reproducible in larger international multi-centre trial. We will submit the main results of the trial in an open-access peer-reviewed journal within 3 months after 240th subject completed the 90-day follow up visit, which is expected to happen in Q4 of 2021. We will make fully de-identified record-level raw data available in a public database

ANALYSIS OF POTENTIAL CONFLICT OF INTEREST

This is investigator-initiated trial endorsed by Czech Society of Anaesthesia and Intensive Care. The most significant resource for the study is unpaid voluntary work of all the personnel conducting the study, who decided to do so in times of worldwide pandemic crisis. Part of the resources were gathered from voluntary donors in a crowdfunding campaign conducted by medical student association. Pharmaceutical company Zentiva, ltd. was approached by investigators and kindly agreed to provide study medication at no cost;

however, it has had nor will have any role in study design; collection, management, analysis, and interpretation of data; writing of the report; or the decision to submit the report for publication.

PHARMACOVIGILANCE

The study will be conducted in accordance with the protocol, Good Clinical Practice and current Czech legislation. It is of upmost importance for the safety of all subject in this and other trials that all personnel involved in this trials complies with the KLH-21, version 7, directive of SUKL on Reporting Adverse Reactions to Medicinal Products for Human Use in a Clinical Trial and to Medicinal Products without Marketing Authorisation, issued in accordance with the provisions of Section 56, point 13) Act No 378/2007 Coll., on Pharmaceuticals and on Amendments to Some Related Acts, as amended. In particular, the investigator is obliged to promptly report all suspected unexpected serious adverse events (SUSARs) to the sponsor. A prompt report (no later than within 24 hours from the moment when the investigator has learnt about the fact) will be followed be detailed written reports. In prompt and follow-up reports, subjects are identified using unique numerical codes, which were assigned to the subjects.

Of note, this clinical trial is performed on population of critically ill patients with very high mortality rate. Therefore, if death of a study subject, is deemed by investigator as resulting from the natural course of the underlying disease and no relation to study treatment or procedures is suspected, such an event shall not be considered SAE and reported as such.

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LIST OF APPENDICES

Appendix 1 Specifications of Product Characteristics

Appendix 2 Informed Consent Form