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Protocol Title

"COLCHICINE TO COUNTERACT INFLAMMATORY RESPONSE IN COVID-19 PNEUMONIA"

Protocol Number: 1.0

Investigational Compound: colchicine

Short Title: ColCOVID-19

Promoter Name and Legal Registered Address: Azienda Ospedaliero-Universitaria di Parma, Via Gramsci 14, 43126 Parma, Italy

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Sommario

1. SYNOPSIS

Rationale

There is currently no approved treatment available for COVID-19 infection. The use of antiviral drugs Lopinavir, Ritonavir, Darunavir gave It has multiple drug-to-drug interactions with many commonly used drugs in clinical practice; thus, its clinical safety is not determined.

The recommendation for using CLC as treatment of COVID-19 is based on the hypothesis that colchicine controls the cytokine storm occurring during COVID-19 infection by modulating NLRP3 inflamma some activity.

Colchicine (CLC) is an old, low cost and easily available drug with an acceptable known clinical tolerability and safety profiles.

Objectives and Endpoints

Objectives	Endpoints
Primary	
To evaluate the clinical efficacy of colchicine relative to the control arm in adult patients hospitalized for COVID-19 with pneumonia and clinically stable conditions	Time to clinical improvement: defined as time from randomization to an improvement of two points from the status at randomization on a seven-category ordinary scale or live discharge from the hospital (whatever comes first) as recommended by Coronavirus Disease (COVID – 2019) R&D Geneva World Health Organization http://www.who.int/
Secondary	
To describe: - Clinical efficacy of colchicine compared to the control arm by clinical severity - Duration of predefined symptoms and signs (if applicable) - Duration of supplemental oxygen dependency (if applicable) - Incidence of new mechanical ventilation use during the study - Duration of new mechanical ventilation use during the Study - Need for admission into intensive care unit (ICU)	1. Clinical status as assessed with 7-category ordinal scale on days 7 and 14 2. Mortality at day 28 3. Duration of mechanical ventilation 4. Duration of hospitalization in survivors 5. Time in days from treatment initiation to death 6. Time to negativization of two consecutive pharyngo-nasal swab 24-72 hrs apart 7. Time to remission of fever in patients with T>37.5°C at enrollment

Study Design

This is a multicenter, randomized, phase 2 study, open-label, enrolling patients with COVID-19 infection with pneumonia and stable conditions.

Treatment and Duration

Colchicine will be administered orally as tablets containing 1mg Colchicine.

Colchicine 1 mg/day (oral administration) from day 0 (day of first administration) to day 21

Schedule of assessments

Procedure	Baseline	Treatment and hospitalization period	Discharge	Follow up (28 day)
Informed consent	X			
Inclusion and exclusion criteria	X			
Demography	X	X		
Comorbidities and coexisting conditions:	X	X		
Clinical parameters:	X	X		
Lab parameters:	X	X		
Pharyngo Nasal swab	X	X		
Imagining: CT scan , thorax X-ray	X	X		
Concomitant drugs (yes/no):	X	X		
Arterial Blood Gas (ABG)	X	X	X	
Respiratory assistance assessment	X	X	X	
Laboratory assessments	X	X	X	
IL-6 and CRP levels	X	X	X	
Vital signs	X	X	X	
PaO2/FiO2, Glasgow coma scale	X	X	x	
Mean arterial pressure,	X	X		
AE review (including SAEs)	X	X	X	X
Concomitant medication review	X	X	X	
Thoracic CT scan or Chest XR if clinically indicated	X		X	
Follow-up information may be collected via telephone calls, patient medical records and/or clinical visits				X

2. INTRODUCTION

2.1 Background

Beginning in December 2019, a novel coronavirus, designated SARS-CoV-2, has caused an international outbreak of respiratory illness termed Covid-19. The full spectrum of COVID-19 ranges from mild, self-limiting respiratory tract illness to severe progressive pneumonia, multiorgan failure, and death. Thus far, there are no specific therapeutic agents for coronavirus infections.

Cytokines and chemokines are thought to play an important role in immunity and immunopathology during virus infections [3]. Patients with severe COVID-19 have higher serum levels of pro-inflammatory cytokines (TNF-α, IL-1 and IL-6) and chemokines (IL-8) compared to individuals with mild disease or healthy controls, similar to patients with SARS or MERS [3]. The change of laboratory parameters, including elevated serum cytokine, chemokine levels, and increased NLR in infected patients are correlated with the severity of the disease and adverse outcome, suggesting a possible role for hyper-inflammatory responses in COVID-19 pathogenesis. Importantly, previous studies showed that viroporin E, a component of SARS-associated coronavirus (SARS-CoV), forms Ca2C-permeable ion channels and activates the NLRP3 inflammasome (1). In addition, in SARS-CoV Viroporin 3a Activates the NLRP3 Inflammasome (2). The mechanisms are unclear.

Colchicine (CLC), an old drug used in auto-inflammatory disorders (i.e., Familiar Mediterranean Fever and Bechet disease) and in gout, counteracts the assembly of the NLRP3 inflammasome, thereby reducing the release of IL-1b and an array of other interleukins, including IL-6, that are formed in response to danger signals. Recently, colchicine has been successfully used in two cases of life-threatening post-transplant capillary leak syndrome. These patients had required mechanically ventilation for weeks and hemodialysis, before receiving colchicine, which abruptly restored normal respiratory function and diuresis over 48 hrs [4].

2.2 Rationale for the study

There are no approved treatments for COVID-19 at this time. A recent clinical trial failed to show a significant benefit of currently used antivirals lopinavir-ritonavir over support therapy alone in patients with advanced disease. There is also no evidence so far of the benefit of darunavir-cobicistat which share with lopinavir-ritonavir the same mechanism of action of. In theory, these could be effective in restrain viral infection when given in the early phase of the infection, but they have significant interactions with the absorption and metabolism of other commonly used medications. Results from trials from other anti-viral drugs such as remdesivir are ongoing. Pending the results of those studies, hydroxychloroquine is currently the most widely used antiviral drugs.

Anyhow, it is likely that some patients additionally benefit from the use of anti-inflammatory drugs that counteract the hyper-inflammatory phase of the disease.

The use of colchicine is recommended for treatment of COVID-19 patients because it may reduce the “cytokine storm” occurring during the late phase of the disease. Colchicine inhibits the assembly of the NLRP3 inflammasome, and, therefore, reduces the release of IL-1b and other interleukins, including IL-6. The excessive production of these cytokines might cause severe pneumonia that can progress to life-threatening acute respiratory distress syndrome.

Colchicine is a well-known, available, cheap, well tolerated and safe drug that has been used for years. It has not significant drug interactions with hydroxychloroquine, a widely used antimalarial drug with antiviral effect. Rather, colchicine (anti-inflammatory drug) and hydroxychloroquine (antiviral drug) could exert a synergistic action against the disease. The same holds true for remdesivir.

There or going trials on various alternative drugs for the treatment of the hyper-inflammatory phase of the disease (such as anti-IL6 tociluzimab). However, they are not available as yet, and it is likely that their indications will eventually be restricted to the most severe forms of the disease. We therefore designed this study to target in-hospital patients with COVID-19 and pneumonia at low and intermediate-risk and in stable

clinical conditions, that may be less likely to be candidates to the newer drugs that will be available in the future. Indeed, at the University Hospital of Parma, because of the lack of therapeutic options and/or the non-availability of anti-inflammatory drugs to COVID-19, we had started using colchicine in both outpatients and inpatients with COVID-19 on over 50 subjects since March 15th, 2020. Those subjects are now being retrospectively follow-up by an ongoing study (approved by the AVEN Ethics Committee on 31/03/2020 with prot. n. 13306.).

3. OBJECTIVES AND ENDPOINTS

3.1 Primary objectives

To evaluate the clinical efficacy of colchicine relative to the control arm in adult patients hospitalized for COVID-19 with pneumonia and clinically stable conditions

3.2 Secondary objectives

To describe:

- Clinical efficacy of colchicine compared to the control arm by clinical severity
- Duration of predefined symptoms and signs (if applicable)
- Duration of supplemental oxygen dependency (if applicable)
- Incidence of new mechanical ventilation use during the study
- Duration of new mechanical ventilation use during the Study
- Need for admission into intensive care unit (ICU)
- Evaluate duration of hospitalization (days)
- Evaluate the 28-day mortality rate

4. STUDY DESIGN

This project is written at the time of the COVID-19 pandemic and while the number of people who get infected or is hospitalized or die for respiratory complication is dramatically increasing. There is currently no approved treatment available for COVID-19 infection.

4.1 Type of study

This is a multicenter, randomized, phase 2 study, open-label, enrolling patients with COVID-19 infection. Study drug: colchicine + standard of care versus standard of care.

4.2 End of Study Definition

A participant is considered to have completed the study if he/she has completed the last scheduled procedure shown in the Schedule of assessments. The end of the study is defined as the date of the last scheduled procedure shown in the Schedule Assessments for the last participant in the trial. Planned duration of the study is 3 months.

5. STUDY POPULATION

5.1 Inclusion criteria

- Patients aged 18 years or above
- Belonging to the low- to intermediate-risk strata according to the criteria of the Emilia-Romagna Region, Italy (PG/2020/0240975 del 21 marzo 2020 “Protocollo terapeutico per la terapia antivirale

per I pazienti con infezione da Covid19", Scenario 2 and 3A, in a scale of increased levels of severity) and to the SIMIT guidelines (http://www.simit.org/IT/formazione/linee-guida.xhtml)), that is patients in stable medical conditions (MEWS<3, see Table below) with the following characteristics:

- Pauci-symptomatic with positive nasopharyngeal swab for COVID-19 + age ≥70 years and/or clinical risk factors for poor outcome (clinically relevant chronic lung disease, diabetes and/or heart disease) + even minimal CT scan findings (>5% of lung parenchima) suggestive of viral pneumonia (ground-glass opacities and/or patchy consolidation, and/or interstitial changes with a peripheral distribution)

- Symptomatic (temperature ≥38°C and/or intensive cough and/or shortness of breath), + CT imaging showing typical findings of viral pneumonia (ground-glass opacities, multifocal patchy consolidation, and/or interstitial changes with a peripheral distribution) and positive or pending pharyngo-nasal swab for COVID-19.

Table. MEWS Scale

Blood Pressure -mmHg	≤70	71-80	81-100	101-199		≥200		
Heart Rate –rpm		<40	41-50	51-100	101-110	111-129	≥130	
Respitariory Rate - rpm		<9		91-14	15-20	21-29	≥30	
Temperature -°C		<35	35-38.4			≥38.5		
Consiuousness				fully alert	verbal response	response to pain	non-responsive	
Score	**3**	**2**	**1**	**0**	**1**	**2**	**3**	**Total**

5.2 Exclusion criteria

- Unstable clinical conditions (MEWS≥3)
- Respiratory rate > 30 rpm, PaO2/FiO2 < 200mmHg
- Pregnant or breast feeding
- Hepatic failure Child-Pugh C
- Enrollment in other pharmacological studies

Treatment with
- Chronic treatment with colchicine
- *Ongoing* treatment with antiviral drugs that include ritonavir or cobicistat (*Previous* treatment with antiviral drugs that include ritonavir or cobicistat is NOT an exclusion criteria)
- Any medical condition or disease which in the opinion of the Investigator may place the patient at unacceptable risk for study participation.

5.3 Removal of patients from therapy of assessment

For secondary endpoints, investigators must clearly distinguish between study drug treatment discontinuation and study withdrawal. Patients who discontinue study drug treatment of who initiate medication changes (including those prohibited by the protocol) will not be automatically withdrawn from the study, but all efforts must be made to continue to follow patients for all scheduled visits.
Patients may be withdrawn from the study for only one of the following two reasons:

1. Patient withdrawal of consent to contribute additional outcome information;
2. Loss to follow-up

Patients may discontinue study drug treatment for any of the following reasons:

1. Patient withdrawal of consent
2. The investigator may discontinue study drug treatment if, in his/her clinical judgment, it is in the best interest of the patient;
3. The promoter may request discontinuation of the drug for safety reasons.

If the patient develop a Grade 3 or higher adverse event considered possibly related to study medication (CLC), the study medication needs to be suspended, and may only be restarted if the event has resolved and the investigator considers it appropriate to do so.

5.4 Screen failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomly assigned to study intervention. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure reasons, eligibility criteria, and any serious adverse event (SAE).
Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened. Rescreened participants should be assigned a different participant number.

6. STUDY MEDICATION/TREATMENT

6.1 Product Characteristics

Colchicine will be administered orally as tablets containing 1mg Colchicine. The tablets are manufactured under current good manufacturing practice. All doses of study medication will be administered orally and supplied by the local hospital pharmacy

6.2 Treatment Compliance

The effective doses of colchicine received by each participant during the study will be recorded.

6.3 Concomitant Therapy

Concomitant treatment with antiviral drugs (Lopinavir, Ritonavir, Darunavir) is not allowed.
There is no contraindication to concomitant treatment with Hydrossycloroquine or remdesivir
In case of suspected or demonstrated concomitant infections that can be successfully treated with antimicrobials in order to make the patient eligible, such treatments are allowed.
However, any medication that the participant is receiving at the time of enrollment or receives during the study will be recorded along with:
- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

6.4 Treatment protocol

Colchicine can be administered orally along with hydroxychloroquine at the usual dosage for both drugs. Hydroxycloroquine is usually given at the dosage of 200mg b.i.d. or t.i.d, with or without an initial loading dose of 400mg.

6.4.1. Colchicine dosage

Colchicine 1 mg/day (oral administration) from day 0 (day of first administration) to day 21,

6.4.2. Colchicine dosage adjustement

- to be reduced to 0.5/day if the patient develops severe diarrhea.
- Acute or Chronic kidney failure: Patients not requiring dialysis, eGFR < 30mL/min: 0.5 mg/day
- Patients requiring dialysis: 0.5 mg every other day, after each dialysis session

- Patients with advanced liver impairment (up to Child-Pugh score B): 0.5 mg every other day

6.4.3. Standard of care

Standard of care include the following:

- Oxygen supply or non-invasive ventilation to target a peripheral blood saturation > 94%
- Hydroxychloroquine 200mg b.i.d w/o an initial 1-2 days loading dose of 400 mg bid for 1-2 days (the dose may be reduced in patients with advanced CKD according to local protocols) or remdesivir 200 mg in.v on day 1, followed by a 100,g q.d.
- Previous treatment (usually for 5 days) with antiretroviral, either with lopinavir/ritonavir or darunavir/cobicistat si permitted
- Azithromycin 500 mg q.d., usually for 5 days, is allowed in patients with pneumonia (even in association with hydroxychloroquine)
- Prophylaxis for deep vein thrombosis as for local protocols
- Steroids are not routinely recommended but may considered in unstable patients as for local protocols

7. STUDY ASSESSMENTS AND PROCEDURES

Screening evaluations must be completed and reviewed to confirm that potential participants meet eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
Procedures conducted as part of the participant's routine clinical management (eg, blood count) and obtained before informed consent may be used for screening or baseline purposes provided the procedures met the protocol-specified criteria.

7.1 Screening procedures

- Informed Consent Form
- Demographics: Age, sex, ethnicity
- Comorbidities and coexisting conditions: diabetes, hypertension, cancer, Chronic Kidney Disease (CKD), transplantation
- Clinical parameters: Respiratory rate (rpm), Heart rate (rpm), Blood pressure (mmHg), PO2/fiO2, temperature, dyspnea, diarrhea, myalgias, Acute Kidney Injury (AKI)
- Lab parameters: IL-6 (pg/mL), CRP (mg/L), procalcitonin (ng/mL), BNP (pg/mL), s-Creat (mg/dL) , PTL (x10^3/mmc), WBC(x10^3/mmc), Lymphocytes (cells/mmc), Hb (g/dL), d-dim (ng/mL), AST(IU/mL), ALT (IU/mL), LDH (IU/mL), CPK (IU/mL), ferritin (ng/mL), albumin (g/dL)
- Pharyngo Nasal swab: positive or pending
- Imagining: CT scan , thorax X-ray
- Concomitant drugs (yes/no): Antibiotics, hydroxychloroquine, Angiotensin Converting Enzyme-Inhibitors (ACE-I), (Angiotensin receptor Blockers) ARB

7.2 Treatment and procedures during hospitalization period

Time schedule: Day 0, +1, +2, +3, +4-
Arterial Blood Gas (ABG)
- Respiratory assistance assessment
- Laboratory assessments: At least blood count, bilirubin, AST, ALT, creatinine, PT, PTT, LDH, D-dimer, bilirubin, platelet and creatinine levels
- IL-6 and CRP levels
- Vital signs (respiratory rate, pulse, blood pressure and temperature) will be obtained as appropriate
-PaO2/FiO2, Glasgow coma scale
- Mean arterial pressure,

- AE review (including SAEs)
- Concomitant medication review

7.3 Procedures before discharge or day +7, +14, +21

- Arterial Blood Gas (ABG) Analysis
- Respiratory assistance assessment
- Laboratory assessments: At least blood count, bilirubin, AST, ALT, creatinine, PT, PTT, LDH, D-dimer, bilirubin, platelet and creatinine levels
- IL-6 and CRP levels
- Vital signs (respiratory rate, pulse, blood pressure and temperature) will be obtained as appropriate
-PaO2/FiO2 mean arterial pressure, and
- Thoracic CT scan or Chest XR if clinically indicated
- AE review (including SAEs)
- Concomitant medication review

7.4 Follow up (28 days) procedures

- Follow-up information may be collected via telephone calls, patient medical records and/or clinical visits
- AE review (including SAEs)

7.5 Efficacy assessment

7.5.1. PaO2/FiO2 ratio (P/F)

PaO2/FiO2 ratio represents the ratio between the arterial blood partial pressure of the oxygen (PaO2) and the percentage of oxygen supplied (fraction of inspired oxygen, FiO2). This parameter is calculated from arterial blood gas analysis and is commonly used for the definition of ARDS. A P/F ratio of 300 to 200, indeed, identifies Mild ARDS, 200 to 100 Moderate ARDS, and a respiratory failure featuring a P/F less than 100 is suggestive for Severe ARDS.

7.5.2. Seven category ordinary scale (6):

1. not hospitalized with resumption of normal activities
2. not hospitalized but unable to resume normal activities
3. hospitalized not requiring supplemental oxygen
4. hospitalized, requiring supplemental oxygen
5. hospitalized, requiring nasal high flow oxygen therapy, noninvasive mechanical ventilation or both
6. hospitalized, requiring ECMO, invasive mechanical ventilation or both
7. death

7.5.3. Laboratory assessment

Lymphocyte count, C-reactive protein (CRP) are assessed by routinely used determination of blood count and CRP. IL-6 levels will be assessed using commercial ELISA method.

7.6 Adverse Events

7.6.1. Definitions

An adverse event (AE) is any untoward medical occurrence in a study participant administered the medicinal products and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease

temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.
An adverse reaction (AR)is an untoward and unintended response to the investigational medicinal products related to any dose administered, judged by either the investigator or the promoter.
An unexpected adverse reaction (UAR) is an adverse reaction, the nature or severity of which is not consistent with the applicable products information (investigator's brochure).
A Serious Adverse Event (SAE) is untoward medical occurrence or effect that at any dose results in death, risk of death, permanent disability/incapacity, hospitalisation or prolongation of existing hospitalization or need for urgent medical treatment, or another medically important serious event as judged by the investigator. Further, any unexpected changes in relation to the toxicity profile of the drugs used of grade 3, as well as adverse event(s) which, although not falling within this definition, are considered unexpected and serious by the Investigator should be reported. The causality of SAEs (their relationship to all study treatment/procedures) will be assessed by the
investigator(s) and communicated to the responsible of the Farmacovigilance. A Suspected Unexpected Serious Adverse Reaction (SUSAR) is an unexpected adverse reaction judged serious by the Investigator and/or Promoter, that is not consistent, either in nature or in severity, with the applicable product information.

7.6.2. Collection and reporting of adverse events

All adverse events recorded from time of signature of informed consent, throughout the treatment and observation period up to 30 days following registration, have to be reported in the toxicity case report form, graded according to the corresponding CTCAE term (Version 5.0). An external CRO will be in charge for the system for Adverse Effect Drug reporting.
The Investigator must immediately report to the promoter all serious adverse events. The report should be made using the SAE report form to the coordinating office immediately and not exceeding 24 hours following knowledge of the event. All SAE must be also reported in the toxicity case report form within the corresponding CTCAE term.
During the course of the study all AEs and SAEs should be proactively followed up for each subject. Every effort should be made to obtain a resolution for all events, even if the events continue after discontinuation/study completion. The investigator is responsible for following all SAEs until resolution, until the subject returns to baseline status, or until the condition has stabilized with the expectation that it will remain chronic, even if this extends beyond study participation.

7.6.3. Causality assessment between treatment and event

The following criteria will be used for causality assessment:
Term Description

- CERTAIN A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals.
- PROBABLE/ LIKELY A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to the concurrent disease or other drugs or chemicals.
- POSSIBLE A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals.
- UNLIKELY A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.
- NOT RELATED There is no causal relationship between the treatment and the event
- CONDITIONAL/UNCLASSIFIED A clinical event, including laboratory test abnormality, reported as an

adverse reaction, about which more data is essential for a proper assessment or the additional data are under examination.

- UNASSESSIBLE/UNCLASSIFIABLE A report suggesting an adverse reaction which cannot be judged because

information is insufficient or contradictory, and which cannot be supplemented or verified.

7.6.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the promoter of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation are met.
- The promoter will review all adverse events and issue queries directly to the Investigator reporting the event. The promoter will determine if an event qualifies as a SUSAR.
- The Reference Safety Information (RSI) necessary to classify an adverse reaction as SUSAR, based on the nature and seriousness, including the frequency, is located in the specific section of the Investigator's Brochure of Colchcicine (section 4.8 as of the version released in May 2019).
-The promoter has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation.
The promoter will report all SUSARs to Eudravigilance through the EVCTM, to all participating Investigators, to the Local Ethical Committee and to the manufacturer within the timelines of the article 17 of the European Directive 2001/20/EC.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information (eg, summary or listing of SAEs) from the promoter will review and then file it along with the Investigator's Brochure.
- The promoter will provide an annual Development Safety Update Report, including all Serious Adverse Events occurring in the Study, to the Regulatory Agency, all participating Investigators, and to the Ethical Committees of participating centers.
- The Investigators are responsible for informing their Ethics Committee of the SAE reported
in their centre, as per local requirements.

7.6.5. Safety Assessments

Planned time points for all safety assessments are provided in the schedule of assessments table.
Pharmacovigilance activities for the study shall be carried out by a third company, under a specific agreement to be stipulated, to ensure that the Promoter (University Hospital of Parma) complies with current legislation concerning collection and reporting of adverse events/reactions, and meets the set deadlines.

SIDE EFFECTS (from FDA, approved since 2009)
<u>Adverse Effects</u>
>10%
GI effects (eg, diarrhea, nausea, cramping, abdominal pain, vomiting) (26-77%)
1-10%
Fatigue (1-4%)
Gout (0-4%)
Pharyngolaryngeal pain (2-3%)
Headache (1-2%)
<u>Frequency Not Defined</u>
Neuromuscular toxicity (eg, muscle pain, weakness)
Myelosuppression

Disseminated intravascular coagulation Injury to cells in the renal, hepatic, circulatory, and central nervous systems
Postmarketing Reports
Neurologic: Sensory motor neuropathy
Dermatologic: Alopecia, purpura, maculopapular rash, rash
GI: Lactose intolerance, abdominal cramping, abdominal pain, vomiting, diarrhea, nausea
Hematologic: Thrombocytopenia, leukopenia, granulocytopenia, pancytopenia, aplastic anemia
Hepatobiliary: Elevated liver transaminases
Musculoskeletal: Myotonia, muscle weakness, myopathy, elevated creatine phosphokinase, muscle pain, rhabdomyolysis
Reproductive: Azoospermia, oligospermia

8. END POINTS

Primary Outcome
Time to clinical improvement: defined as time from randomization to an improvement of two points from the status at randomization on a seven-category ordinary scale (cfr 7.5.2) or live discharge from the hospital (whatever comes first) as recommended by Coronavirus Disease (COVID – 2019) R&D Geneva World Health Organization http://www.who.int/
Secondary outcomes
1. Clinical status as assessed with 7-category ordinal scale on days 7 and 14
2. Mortality at day 28
3. Duration of mechanical ventilation
4. Duration of hospitalization in survivors
5. Time in days from treatment initiation to death
6. Time for negativeization of two consecutive pharyngo-nasal swab 24-72 hrs apart
7. Time to remission of fever in patients with T>37.5°C at enrollment

9. STATISTICAL CONSIDERATIONS

9.1 Trial procedures

Open label, 1 to 1, individually randomly assigned, no placebo/blinding due to the emergency nature of the trial. However, assessment of the 7 category-ordinal scale will be carried out by a researcher blinded to treatment assignment who will review dedicated data-collection forms reporting daily respiratory parameters and management of respiratory failure.
Study drug + standard care vs standard care alone
Study drug: colchicine
To balance the distribution of oxygen support within the two groups as indicator of severity of respiratory failure randomization will be stratified on the basis of respiratory support at the time of enrollment (stage 4 and Stage 5 in the seven category ordinary scale)

9.2 Sample size assumption and estimates

Based on data from Cao et al (6),in which the median of the cumulative incidence of improvement rate of the standard of care group is 16 days, we assumed that in our standard of care group the cumulative incidence of improvement rate will be equal to 50% at 14 days, since our target population does not consider the more severe patients as in Cao et al. Basing on that and by referring to the Cao et al results raised-up in the "modified-intention to treat population" analysis (since it could be considered more similar to the our target population), the median improvement in our experimental arm will be approximately 30% shorter. Therefore, we estimated that 310 patients (155 per group) would be required to achieve a 80%

power to detect a median time to improvement of 10 days in the colchicine + standard of care group compared to 14 days in the standard of care group, using a log-rank test with a two-sided alpha level of 0.05, assuming no lost to follow-up after 28 days from recruitment and an enrolment period of 30 days.

9.3 Statistical analysis

Data will be analyzed with an intention to treat basis including all patients who undergo randomization.

Kaplan-Meir curves and log-rank test will be used to assess clinical significance, and Cox multiple regression analysis will be used to adjust for potential imbalance in baseline prognostic indicators (confounders). Therefore, the estimate for the difference in the primary endpoint will be reported as hazard ratio. Hazard ratio will be reported along with the associated 95 percent confidence interval.
Plots will be presented with the cumulative incidence of the outcome in both groups, to show the difference between the treatment arms at each time point.

Baseline confounders for multiple regression analysis will be as follows: age (yrs), sex (indicator variable for male), body mass index (Kg/m^2), baseline seven category ordinal scale (see), treatment with hydroxychloroquine (indicator variable), CKD, hypertension, diabetes, and cancer as comorbidities (one indicator variable for each), use of daily steroids (indicator variable for >0.05mg/kg/day prednisone equivalents), transplantation status (indicator variable), time elapsed from nasopharyngeal swab to enrolment (days), CT scan pneumonia extension ad admission (percentage).

Difference in secondary outcomes will be reported as 95% confidence intervals without any attempt to adjust for multiplicity. Subgroup analysis, presented as confidence intervals as opposed to statistical test, will be performed in patients stratified by the Seven Category Ordinary Scale (whether or not category 4 or lower), age (whether or not age below 80 yrs), sex, presence of comorbidities, treatment with hydroxychloroqune, time elapsed from nasopharyngeal swab to enrolment (whether less than five days), CT pneumonia extension (whether or not lower than 50%).

Secondary outcomes will be analyzed as follows:
Clinical status as assessed with 7-category ordinal scale on days 7 and 14 will be examined by ordinal logistic regression. We will use Brant test to check that proportional odds assumption holds, otherwise we will employ multinomial logistic regression.
The odds ratio from the ordinal regression models can be interpreted in a similar way as that of the binary logistic regression, namely the relative increase in the probability (odds) of belonging to one 7-category ordinal scale category given the treatment with colchicine + standard of care vs standard of care. However, unlike binary logistic regression, where the reference category would be the “not hospitalized with resumption of normal activities”, in ordinal logistic regression the reference is the lower 7-category ordinal scale, therefore the odds ratio is the relative probability (odds) of belonging to the higher 7-category ordinal scale given treatment assignment to the colchicine + standard of care arm. To make the interpretation of the ordinal regression model easier, we will plot the fitted predicted probability for each one of the 7-category ordinal scale, given that the patient is assigned to the colchicine + standard of care or to the standard of care arm. Adjusting for the same confounding factors considered for primary endpoint will be carried out by fitting multivariable ordinal logistic regression models.
Mortality at day 28. Difference will be tested by two-sided Fisher’s exact test and the 95 percent confidence interval for the risk difference calculated using exact confidence intervals. Adjustment for the same confounding factors considered for primary endpoint will be carried out by fitting multivariable models. To this purpose, we will use generalized linear models for binomial outcome and identical link to estimate adjusted risk differences and 95 percent confidence intervals. If these models fail to run, a logistic regression will be used.

Duration of mechanical ventilation will be analyzed tested by Mann-Whitney test. Adjusted differences will be analyzed by negative binomial regression via generalized linear models, adjusting for the same confounding factors considered for primary endpoint in negative binomial multivariable regression models.
Duration of hospitalization in survivors will be analyzed by negative binomial regression via generalized linear models, adjusting in multivariable models for the same confounding factors considered for primary endpoint.
Time in days from treatment initiation to death will be analyzed by Kaplan-Meier plots and by Cox-regression analysis as for the primary endpoint.
Time to negativization of two consecutive pharyngo-nasal swab 24-72 hrs apart will be tested by Mann-Whitney test. Adjustment for confounders will be carried out using methods by interval-censored data based parametric multiple regression models (e.g. Weibull).
Time to remission of fever (days) in patients with T>37.5°C at enrollment will be analyzed by Kaplan-Meier plots and by Cox-regression analysis as for the primary endpoint.

10. ETHICS, QUALITY ASSURANCE AND MONITORING

The procedures set out in this study protocol are designed to ensure that the promoter and the Investigators abide by the principles of the Good Clinical Practice guidelines of the International Conference on Harmonization (ICH) and the Declaration of Helsinki in the conduct, evaluation and documentation of this study. The study will be carried out adhering to local legal requirements and the applicable national law, whichever represents the greater protection for the individual. Study protocol, patient information and informed consent will be submitted to the appropriate
Ethical Committee for approval. The promoter will inform the appropriate Ethical Committee about any changes in the study protocol which could interfere with the patient's safety. The monitoring activities during pandemia will be primarily or exclusively performed without peripheral visits. Remote monitoring will be performed through periodic, comprehensive connections through the web or the telephone by promoter personnel or representatives.

10.1 Informed Consent Process

The physicians treating the hospitalized patient are responsible for information of the patient and obtaining of the Informed Consent. The consent can be oral if a written consent cannot be expressed. If the subject is incapable of giving an informed consent and an authorized representative is not available without a delay that would, in the opinion of the Investigator, compromise the potential life-saving effect of the treatment this can be administered without consent. Consent to remain in the research should be
sought as soon the conditions of the patient will allow it.
The same procedure apply to the information of the patient and providing of consent to the processing of personal data according to the European Regulation n. 679/2016 on the Protection of Personal Data, the Personal Data Protection Code (Legislative Decree 196/03) and subsequent amendments and additions, and to the provisions, guidelines and general authorizations of the National Guarantor for Personal Data Protection.

11. DATA MONITORING COMMITTEE

An Independent Data Monitoring committee (IDMC) will be nominated to warrant the quality of the study management and analysis. The IDMC will be made of 3 to 5 members, selected among statisticians, trialists and experts in Infectivology and Resuscitation; the IDMC will be nominated after the list of participating

Institutions will be definitive, to select among experts not directly involved in the study. An IDMC charter will be produced after the nominations.
The IDMC will be responsible for:

- reviewing activity and safety data through progress reports produced by the promoter and recommending for example modifications in case of unexpected or unexpectedly severe toxicities for study treatment, or in case of preliminary data suggesting inactivity or surprisingly positive efficacy in specific subgroups of patients. These corrections may be modifications of the treatment, the inclusion criteria or conditions for retreatment, or the sample size, or the study procedures or early study termination.
- evaluating the effect on the study of possible changes in scientific evidence, such as results of other studies, and recommending modifications as above on the basis of such external data.

Considering the setting of the present study, which apply to a health emergency situation, progress report will be produced weekly and the IDMC will examine all the reports produced, in collaboration with the steering committee and/or within closed meetings, and will suggest possible modifications as described above.

12. DATA COLLECTION PROCEDURES

The study's Principal investigator (PI) is responsible for data collection, handling and storing. For each subject enrolled in the study, an Electronic data collection form (electronic Case Report Form – eCRF) will be completed, containing an identification code and all necessary information for end point measurement. The data collection form is developed in compliance with the minimization principle, i.e. it only comprises data that are strictly necessary to fulfill the study's primary and secondary objectives. Sensitive/particular data (first and last name, date of birth, etc…) that are only available in the list kept by the PI will not be collected. The validated eCRF will be developed by a third company to ensure compliance with all criteria set forth in the EMA/FDA guidelines. eCRFs will be made available for review by designed representatives in case of monitoring visits. The researcher shall also allow representatives of regulatory bodies to review the data reported in the eCRF and source documents, in compliance with current legislation.

13. STUDY MONITORING

Monitoring activity will be ensured by the Clinical Trial Quality Team (CTQT) of the University Hospital of Parma, established with Deliberation no.317 of May 4th 2018, with the collaboration of expert monitors of an external CRO for non-profit studies promoted by the University Hospital of Parma. The Research and Innovation Unit of the University Hospital of Parma shall ensure data quality by enacting activities such as data quality assurance and data quality control.

14. ADMINISTRATIVE ASPECTS

This is a non-profit investigator initiated trial. Study protocol, patient information, and informed consent at beginning and at each required amendment will be submitted to the appropriate Ethical Committee for approval.
Coverage for any damage resulting from the participation of the subjects in the clinical trial is included in the general insurance of the individual participating clinical centers.

15. COORDINATING CENTRE CONTACTS

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