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Short Title: **Colchicine in COVID-19: a Pilot study**
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Under the auspices of the Italian Society of Rheumatology (SIR), the Italian Society of Infectious and Tropical Diseases (SIMIT) and by the Italian Thoracic Society (AIPO)

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Approval date: 11/04/2020

PROTOCOL AUTHORIZATION PAGE

I have read this study protocol and agree that it contains all the information required to conduct the study. I agree to conduct the study as set out in this protocol. In particular, I agree to adhere to the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, the guidelines on Good Clinical Practice and the appropriate national laws.

Local Investigator

_____ _____

Date

Trial Promoter Coordinating Centre

_____ _____

Date

Contents

1. Synopsis

Protocol Title: "Treatment with COLchicine of patients affected by COVID-19: a Pilot study"

Short Title: Colchicine in COVID-19: a Pilot study

Rationale

1.1 Introduction

The outbreak of the 2019-coronavirus (SARS-CoV-2) has posed the world at a pandemic risk. The disease (COVID-19) is characterized by an acute respiratory distress and there is an immediate need for any agent before the development of a vaccine. So far, therapeutic regimes include a combination of anti-viral drugs such as lopinavir plus ritonavir. Moreover, preliminary in vitro experiments have suggested that chloroquine/hydroxychloroquine have anti-viral activity. Tocilizumab, an anti-IL-6 monoclonal antibody, has been used with encouraging results in particularly ill patients since there may be a massive release of pro-inflammatory cytokines, especially IL-6, by lung epithelium in severe cases. Indeed, a pro-inflammatory status with high levels of IL-1B, IL-1RA, and TNF-α characterizes the diseases, and higher levels of IL-2, IL-10, and TNF-α have been observed in intensive-care-unit patients. Colchicine is the main treatment of several rheumatic conditions in which the activation of the inflammasome and release of pro-inflammatory cytokines are the key pathogenic events. It alters the organization of actin cytoskeleton by binding to tubulin monomers and inhibits polymer formation. This drug has shown clear anti-inflammatory effects, more pronounced on IL-1/IL-6. Moreover, colchicine has anti-viral properties against flaviviridae and against the recombinant demyelinating strain of mouse hepatitis virus RSA59 as demonstrated reducing virus replication since microtubules are crucial in cell entry and blocking neuronal transport. It inhibits respiratory syncytial virus replication reducing IL-6 and TNF-α levels. A rational of colchicine as an anti-HIV agent has been proposed too. Certain subsets of the 2003-SARS coronavirus replication machinery have been shown to activate the inflammasome and to move in the cell in a manner that corresponds with microtubule-associated transport, inducing the formation of double-membrane vesicles in infected cells. The question may be whether this occurs during SARS-CoV-2 infection and if microtubule disruption may influence viral genome replication levels.

A dysregulated activation and inflammatory activity of myeloid cells is one the main pathogenic events characterizing the infection by coronaviruses. Due to its anti-neutrophilic properties, colchicine was proposed as an anti-viral agent as an adjuvant treatment for RSV bronchiolitis. Thus, colchicine has broad anti-inflammatory effects, anti-viral properties and it is not hampered by an immunosuppressant effect. Unlike other proposed treatments, colchicine is inexpensive and with known side effects.

1.2 Objectives and endpoints

Primary	
To evaluate the efficacy of colchicine by describing:	
1. Rate of entering the critical stage [after 1 month]	Comply with any of the followings: a. Respiratory failure occurs and requires mechanical ventilation; b. Patients combined with other organ failure need ICU monitoring and treatment c. Death
Secondary	
To describe:	
1. Trend of White blood cell count 2. Change of the "Sequential Organ failure Assessment" (SOFA) 3. Rate of biochemical criterion (CK, ALT, ferritin) recovery 4. Rate of disease remission 5. Toxicity of Colchicine	1. White blood cell count 2. "Sequential Organ failure Assessment" (SOFA) score 3. CK, ALT, ferritin 4. Comply with any of the followings: a) No fever, cough and other symptoms; b) SPO2>94% or PaO2/FiO2 >350mmHg without oxygen inhalation 5. Rate of adverse events codified by Common Terminology Criteria for Adverse Events (CTCAE) v5.0

1.3 Overall Design

This is an interventional, pilot, multicenter, randomized, open-label, phase 2 study, enrolling patients with COVID-19 disease.

Phase 2 cohort study

One-month rate of entering the critical stage (either a. Respiratory failure occurs and requires mechanical ventilation; b. Patients combined with other organ failure need ICU monitoring and treatment; c. Death) is the primary endpoint. From available data (1), it can be assumed that 1-month entering the critical stage for the population defined by the selection criteria is around 25% (P0). To verify the hypothesis that the experimental drug may produce a halving of the rate of entering the critical stage (from 25% to 12.5%, P1), 308 patients are needed with an 80% power and a 5% bilateral alpha error. The two arms will be randomized with 154 patients treated with the current treatment and 154 with colchicine added to the current treatment.

1.4 Treatment and Duration

Participants will receive Colchicine 0.5 mg three times a day if weight is less than 100 kg; 1 mg twice a day if weight is more than 100 kg. Maintain if no response is obtained or reduce based on gastrointestinal symptoms appearance at discretion of the Investigator.
Such dose is the same approved by EULAR for the treatment of Gout and FMF.
Colchicine may cause gastrointestinal side effects, particularly diarrhea, in about 9.6% of patients that usually do not require treatment discontinuation.

Procedure	**Baseline before first colchicine administration (possibly no more than 1 day before)**	**Every-day while hospitalized**	**Discharge**	**Follow-up on Day 30**
Informed consent	X			
Inclusion and exclusion criteria	X			
Demography	X			
Full physical examination including height and weight	X			
Medical history (includes past and current medical conditions, and substance usage)	X			
Arterial Blood Gas (ABG) Analysis [1]	X	X	X	
Respiratory assistance assessment	X		X	
Laboratory assessments[2]	X	X	X	
12-lead ECG	X		X	
Vital signs	X	X	X	
SOFA score[3]	X	X	X	
Thoracic CT scan or Chest XR[4]	X		X	
AE review	X	X	X	X
Concomitant medication review	X	X	X	
Survival follow-up	X		X	X

[1]at least one every three day while hospitalized
[2]At least once every three day blood count, bilirubin, AST, ALT, creatinine, ferritin LDH, CRP
[3]SOFA score is calculated considering PaO2/FiO2, Glasgow coma scale, mean arterial pressure, and bilirubin, platelet and creatinine levels.
[4]Radiological evaluation is optional.

2. Introduction

Colchicine is an inexpensive, orally administered, potent anti-inflammatory medication that was initially extracted from the autumn crocus and has been used for centuries. Its mechanism of action is through the inhibition of tubulin polymerization and microtubule generation and, possibly, effects on cellular adhesion molecules, inflammatory chemokines, and the inflammasome (2, 3). Colchicine is currently indicated for the treatment of gout, familial Mediterranean fever, and pericarditis (4-6).

3. Background

The outbreak of the 2019-coronavirus (SARS-CoV-2) has posed the world at a pandemic risk. The disease (COVID-19) is characterized by an acute respiratory distress and there is an immediate need for any agent before the development of a vaccine. Coronavirus-19 disease (COVID-19) is an infectious disease caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), a Betacoronavirus that belongs to the same family as SARS and MERS, which causes pneumonia, requires intensive care unit hospitalization in about 10% of cases and can lead to a fatal outcome (7-10).

To date, there is no registered molecule for the treatment of COVID-19 patients. However, there are ongoing trials on the use of some antivirals and immunosuppressive or immunomodulating drugs that have demonstrated efficacy on COVID-19 both in vitro and on animal models as well as in small cases series.

Certainly, previous experiences on viruses belonging to the same Betacoronavirus family have formed the cornerstones of the current therapeutic strategy (11, 12). The emergency facing the scientific community in addressing the pandemic from COVID-19 provides the rationale for the use of drugs that have not yet been approved and with still preliminary scientific evidence.

So far, therapeutic regimes include a combination of anti-viral drugs such as lopinavir plus ritonavir. Moreover, preliminary in vitro experiments have suggested that chloroquine/hydroxychloroquine have anti-viral activity. Tocilizumab, an anti-IL-6 monoclonal antibody, has been used with encouraging results in particularly ill patients since there may be a massive release of pro-inflammatory cytokines, especially IL-6, by lung epithelium in severe cases (13). Trials to test the efficacy of Tocilizumab on severe COVID-19 patients are being tested in China and in Italy (14, 15).

Indeed, a pro-inflammatory status with high levels of IL-1B, IL-1RA, and TNF-α characterizes the diseases, and higher levels of IL-2, IL-10, and TNF-α have been observed in intensive-care-unit patients (16). Authors recently documented that SARS-CoV and its accessory protein are potent activators of pro-IL-1β gene transcription and protein maturation, and thus are able to activate the NLRP3 inflammasome. Colchicine is the main treatment of several rheumatic conditions (17-19) in which the activation of the inflammasome and the release of pro-inflammatory cytokines is the key pathogenic event. It alters the organization of actin cytoskeleton by binding to tubulin monomers and inhibits polymer formation (20).

3.1 Rationale for Colchicine in COVID-19 infection

Colchicine drug has shown clear anti-inflammatory effect more pronounced on IL-1/IL-6, as evident from *in vivo* studies at therapeutic dosage (18). For instance, Colchicine has demonstrated a protective effect after myocardial infarction at only 0.5 mg/day in terms of incidence of strokes and urgent hospitalizations for angina leading to coronary revascularization (19). Colchicine reduced the C-reactive protein level in these patients similarly to an anti-IL-1 drug, canakinumab. Colchicine is effective in preventive atherosclerosis in multiple ways (21). Indeed, it is highly concentrated in neutrophils and macrophages prolonging its action (22). Its ability to bind tubulin achieves multiple cellular actions, including inhibition of the assembly of the NLRP3 inflammasome. In addition to

its effect on neutrophils (23, 24), colchicine impairs the release of IL-1b into NETs (25). Many of the actions have been demonstrated in patients with coronary disease (26, 27).

Colchicine blocks the NLRP3 inflammasome, a cytosolic complex responsible for the production of IL-1β and IL-18. In patients with acute coronary syndromes, in which interleukin (IL)-1β, IL-18, and downstream IL-6 that are key inflammatory cytokines in the pathogenesis of this condition, colchicine administration was able to significantly reduce transcoronary gradients of all 3 cytokines in by 40% to 88% (27). A similar reduction in IL-6 but also in IL-8 or TNF-alpha levels can be observed in mucocutaneous Behçet's disease patients (28).

Moreover, colchicine has anti-viral properties against flaviviridae and against the recombinant demyelinating strain of mouse hepatitis virus RSA59 as demonstrated reducing virus replication since microtubules are crucial in cell entry and blocking neuronal transport (29, 30). It inhibits respiratory syncytial virus (RSV) replication reducing IL-6 and TNF-α levels (31). A rational of colchicine derivatives as an anti-HIV agent has been proposed too (32). Certain subsets of the 2003-SARS coronavirus replication machinery have been shown to move in the cell in a manner that corresponds with microtubule-associated transport, inducing the formation of double-membrane vesicles in infected cells (33). The question may be whether this occurs during SARS-CoV-2 infection and if microtubule disruption may influence viral genome replication levels (33). As abovementioned, as SARS-CoV, it is likely that also SARS-CoV-2 activates the inflammasome, an effect directly inhibited by colchicine (34).
Moreover, a dysregulated activation and inflammatory activity of myeloid cells is one the main pathogenic events characterizing the infection by coronaviruses (35, 36). Colchicine is a well-known inhibitor of the pro-inflammatory mechanisms induced by neutrophils thus it was proposed as an adjuvant treatment for RSV bronchiolitis (37).

3.2. Benefit/Risk Assessment

Colchicine has broad anti-inflammatory effects, anti-viral properties and is not hampered by an immunosuppressant effect. Colchicine is also inexpensive. Detailed information about the known and expected benefits and risks and reasonably expected adverse events of colchicine may be found in the Investigator's Brochure of the drug.
Colchicine may cause gastrointestinal side effects, particularly diarrhea, in about 9.6% of patients that usually do not require treatment discontinuation.

4. Study Design

4.1. Preamble

This project is written at the time of the coronavirus pandemia and while in Italy the number of people who get infected or is hospitalized for respiratory complication is dramatically increasing. Therefore, the clinical and operational scenario is extremely variable, and it is expected that it will remain so for an unforeseeable time. In addition, very few solid evidences are available on the course of the disease and on the significance of intermediate end-points, before the use of the experimental drug. Therefore, it is accepted in advance that the present protocol may need repeated amendments to comply with evolving knowledge on the pandemia, the rate of complications, and the therapeutic scenario for patients who develop pneumonia. A high degree of adaptivity is therefore planned, that will be strictly discussed with the Independent Data Monitoring Committee that will be nominated soon after the approval of the protocol.

4.2. Overall Design

This is an interventional, pilot, multicenter, randomized, open-label, phase 2 study, enrolling patients with COVID-19 disease.

Phase 2 cohort study
One-month rate of entering the critical stage (either a. Respiratory failure occurs and requires mechanical ventilation; b. Patients combined with other organ failure need ICU monitoring and treatment; c. Death) is the primary endpoint. From available data (1), it can be assumed that 1-month entering the critical stage for the population defined by the selection criteria is around 25% (P0). To verify the hypothesis that the experimental drug may produce a halving of the rate of entering the critical stage (from 25% to 12.5%, P1), 308 patients are needed with an 80% power and a 5% bilateral alpha error. The two arms will be randomized with 154 patients treated with the current treatment and 154 with colchicine added to the current treatment.
Patients' enrollment will start, but may not be limited, from the abovementioned promoting centers.

4.3 Authorship
Besides the abovementioned promoting centers, any center that will enroll at least 10 patients is entitled, in case of publication, of 1 co-author. Any center that will enroll at least 30 patients is entitled, in case of publication, of 2 co-authors. Any center that will enroll at least 50 patients is entitled, in case of publication, of 3 co-authors.

4.4 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 of assessments for the last participant in the trial globally.
In case of sudden worsening and entering the critical stage, patients can be considered as ended the study and any available *rescue therapy* will be adopted.
The trial will be concluded when at least 308 patients will be enrolled and ended the study protocol.

5. Study Population
5.1. Inclusion Criteria
Participants are eligible to be included in the study if the following criteria apply:
1. Any gender
2. ≥18 years old
3. Informed consent for participation in the study (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)
4. Virological diagnosis of SARS-CoV-2 infection (real-time PCR)
5. Hospitalized due to clinical/instrumental diagnosis of pneumonia
6. Oxygen saturation at rest in ambient air ≤94%
7. PaO2/FiO2 ratio of 350 to 200

5.2. Exclusion Criteria
Participants are excluded from the study if any of the following criteria apply:
1. Known hypersensitivity to colchicine or its excipients
2. Severe diarrhea
3. Patients who cannot take oral therapy
4. Pregnant and lactating patients

5. Patients with severe cardiac, renal insufficiency (creatinine clearance (CCL) <30 mL / min)
6. Patients with kidney or liver damage [(AST or ALT> 5 times the normal limits in International Units (ULN)]; or are taking CYP3A4 enzyme - P glycoprotein inhibitors.
7. Known other clinical condition that contraindicates colchicine and cannot be treated or solved according to the judgement of the clinician
8. Neutrophils <1.000 / mmc
9. Platelets <50.000 / mmc
10. Bowel diverticulitis or perforation
11. Patients already in ICU or requiring mechanical ventilation
12. Patients receiving Tocilizumab
13. Patients already enrolled in other clinical trials

6. Treatments

6.1. Treatments Administered

Study Treatment Name:
Colchicine
Dosage formulation:
Colchicina LIRCA
1 mg Unit dose
strength(s)/Dosage level(s):
Colchicine up to 2 mg/day
Such dose is same approved by EULAR for the treatment of Gout and FMF
Route of Administration orally
Dosing instructions:
Packaging and Labeling

Marketing Authorization Holder
ACARPIA Farmaceutici S.r.L,
Via Vivaio, 17 – 20122 Milano
Excipients: lactose, gum arabic, sucrose, magnesium stearate.

6.2. Treatment Compliance

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

6.3. Concomitant Therapy

There is no contraindication to concomitant treatment (excluding tocilizumab, antiviral drugs interfering on CYP3A4 such as lopinavir, ritonavir, darunavir, cobicistat and any drug administered in the course of another clinical trial) that can be defined in advance given the severity of the disease and the availability of few data on
pharmacological interactions of the colchicine schedule planned in this study. Colchicine administration is known to be safe with hydroxychloroquine as well as chloroquine (38, 39).

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

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
- Demography (age, gender, ethnicity)
- Medical history (previous and current diseases, all medications started within 14 days prior to screening visit)
- Full physical examination including height and weight
- Arterial Blood Gas (ABG) Analysis (at least one every three day)
- Respiratory assistance assessment
- Laboratory assessments: at least blood count, bilirubin, AST, ALT, creatinine, ferritin, LDH, CRP levels
- 12-lead ECG
- Vital signs (respiratory rate, pulse, blood pressure and temperature) will be obtained as appropriate
- SOFA score is calculated considering PaO2/FiO2, Glasgow coma scale, mean arterial
- pressure, and bilirubin, platelet and creatinine levels.
- Thoracic CT scan or Chest XR (if clinically indicated)
- AE review (including SAEs)
- Concomitant medication review

7.2. Treatment and procedures during hospitalization period

- Arterial Blood Gas (ABG) Analysis (at least one every three day)
- Respiratory assistance assessment
- Laboratory assessments: At least blood count, bilirubin, AST, ALT, creatinine, ferritin, LDH, CRP levels
- 12-lead ECG
- Vital signs (respiratory rate, pulse, blood pressure and temperature) will be obtained as appropriate
- SOFA score is calculated considering PaO2/FiO2, Glasgow coma scale, mean arterial pressure, and bilirubin, platelet and creatinine levels

- Thoracic CT scan or Chest XR (if baseline evaluation (CT or XR) is available then a reevaluation can be planned after 14 days or at discharge)

7.3. Procedures before discharge

- Arterial Blood Gas (ABG) Analysis
- Respiratory assistance assessment
- Laboratory assessments: At least blood count, bilirubin, AST, ALT, creatinine, ferritin, LDH, CRP levels
- 12-lead ECG
- Vital signs (respiratory rate, pulse, blood pressure and temperature) will be obtained as appropriate
- SOFA score is calculated considering PaO2/FiO2, Glasgow coma scale, mean arterial pressure, and bilirubin, platelet and creatinine levels
- Thoracic CT scan or Chest XR if clinically indicated
- AE review (including SAEs)
- Concomitant medication review

7.4. Follow up (30 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 Assessments

7.5.1. PaO2/FiO2 ratio

PaO2/FiO2 ratio (or P/F ratio for brevity) 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 350 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. Laboratory assessment

Laboratory assessment includes determination of routine parameters (7.1).
Pro-inflammatory cytokines will be evaluated at baseline, at day 6 and at discharge at the centralized laboratory Center for the Study of Rheumatic Diseases, University of Perugia, Strada Vicinale Via delle Corse, Sant'Andrea delle Fratte (PG), Tel. 075 5853540, Fax : 075 5853544, sezione.reumatologia@unipg.it.

7.5.3. Sequential Organ Failure Assessment (SOFA) score

SOFA is a morbidity severity score and mortality estimation tool designed for evaluating organ dysfunction and morbidity. It evaluates 6 variables, each representing an organ system (one for the respiratory, cardiovascular, hepatic, coagulation, renal and neurological systems), and scored from 0 (normal) to 4 (high degree of dysfunction/failure). Thus, the maximum score may range from 0 to 24. The tool can be used for estimating mortality risk.

8. Adverse Events

8.1 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, hospitalization 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 coordinating centre. 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. Adverse events of special interest (AESI)- The following adverse events have been identified as AESI for this study and require prompt reporting to Safety desk for the study immediately and no more 24h of the Investigator becoming aware of the event (expedited reporting), even if the events can be considered non-serious according to the usual regulatory criteria as they may be subject to expedited submission to regulatory authorities:

- Cases of potential drug-induced liver injury (DILI) that include an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's law.
- Suspected transmission of an infectious agent by the study drug (STIAMP), defined as any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, that is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term applies only when a contamination of the study drug is suspected.

8.2. 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).
The Investigator must immediately report to the promoter all serious adverse events. The report should be made using the SAE report form online or by sending the paper copy by fax (+39075045569) 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.

8.3. 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
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

8.4. 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 colchicine (section 6.4.1 as of the version 21 released in September 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 all Ethical Committees of participating centres, 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 centres.
- The Investigators are responsible for informing their Ethics Committee of the SAE reported in their centre, as per local requirements.

8.5. Safety Assessments

Planned time points for all safety assessments are provided in the schedule of assessments table.

9. Statistical Considerations

9.1. Sample Size Determination

The study is designed as a double-arm single-stage phase 2 study with 1-month rate of entering the critical stage as primary endpoint.
Expected 1-month rate of entering the critical stage (P0): 25%
Auspicated 1-month rate of entering the critical stage (P1): 12.5%
Statistical power: 80%
Bilateral alpha error: 5%
Sample size needed: 308 patients

9.2. Randomization

Patients will be randomly assigned to either Colchicine or no investigational treatment according to a randomisation list prepared in advance. Random sequence will be generated using random permuted blocks of unequal length. The randomisation process will be managed by the Epidemiology Research Unit Statisticians of the Italian Society for Rheumatology within the electronic data capture system (REDCap) using its randomization module.

9.3. Populations for Analyses

For purposes of analysis, the following populations are defined:

Population Description

Enrolled	All participants who sign the ICF and are registered
Evaluable	All participants enrolled (Intention-to-treat)
Safety	All participants who take at least 1 dose of study treatment.

9.4. Statistical Analyses

The statistical analysis plan will be developed and finalized before database lock and will describe the participant populations to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. This section is a summary of the planned statistical analyses of the primary and secondary endpoints.
Primary and secondary analyses will be stratified by age categories, gender and eventually other clinically relevant factors (comorbidities, smoke habits etc.).

Endpoint	**Statistical Analysis Methods**
Primary	1-month rate of entering the critical stage is defined as the ratio of patients who will enter the critical stage 1 month from study start out of those registered at baseline and will be described with its 95% confidence interval.
Secondary	All the analysis will be descriptive.

9.4.1. Safety Analyses

All safety analyses will be performed on the Safety Population.

Endpoint	Statistical Analysis Methods
Primary	Toxicity. For each patient and for each type of toxicity described according to CTCAE, the worst degree ever suffered during treatment will be used for descriptive analysis.

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 with all participating centres 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 applies 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 be-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

Patient registration and data collection will be centralized at Centro Studi SIR. Data collection is electronic through the above website (https://redcap.reumatologia.it/index.php) or by paper CRF transmitted by email centrostudisir@reumatologia.it or fax to +39 0287152033 as soon as possible after completion. For contacts for registration and data collection, see contacts page.
Considering the cost "ex factory" of a box of «1 mg pills» containing 60 pills- A.I.C. n. 009964038 (in base 10); class of reimbursement «A»; price (VAT excluded): € 5,30;
The expected cost of the drug in the scenario of treating 154 patients (weight >100kg) for 1 month is: € 816.20

13. Administrative aspects

This is a non-profit investigator initiated trial. In this trial, the experimental drug colchicine will be provided by each participating Hospital.
Study protocol, patient information, and informed consent at beginning and at each required amendment will be submitted to the appropriate Ethical Committee for approval. After the first approval the study will be started at each Italian centre requiring to participate and such participation will be notified together with the approved protocol to the local Institutional Ethical Committee.
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.

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COLVID-19

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