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Protocol Title: "Multicenter study on the efficacy and tolerability of tocilizumab in the treatment of patients with COVID-19 pneumonia"

Protocol Number:3
Amendment Number: 1

Investigational Compound:tocilizumab

Short Title: Tocilizumab in COVID-19 pneumonia

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Approval dateinitial protocol (version 1.3):March 18, 2020

Approval date amendment 1 (protocol version 2): March 24, 2020

Approval date amendment 2 (protocol version 3): April 28, 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

Protocol Amendment Summary of Changes Table

DOCUMENT HISTORY	
Document	**Date**
Amendment 1	*23 March, 2020*
Amendment 2	*20 April, 2020*

Amendment1, 23 March, 2020

Overall Rationale for the Amendment:

The amendment n.1 leading to the version 2 of the protocol is primarily based on what has happened after study opening regarding (1) the enrolment rate, and (2) drug shortage.

Specifically:

1. The study has been opened on the dedicated web platform at 14:00 on March 19, 2020. The sample size of 330 patients enrolled in the phase 2 has been reached in less than 24 hours.

   As a consequence, the coordinating centreoperatively decided:first, to conservatively inflate the recruitment sample size for phase 2 up to at least 400 to allow for possible ineligibility found after registration; later, to also allow the registration of patients potentially eligible for the phase 2 study in the observational prospective cohort (otherwise these patients would have been automatically excludedon the platform due to reached sample size). Such choice was consistent with the rationale of the observational cohort initially planned in the protocol version 1.3.

2. Following registration of participating centres, and enrolment of patients, all the drug that was available in Italy has been distributed by the manufacturer (Roche) to the first 72participating centres.Further drug is going to be distributed by the manufacturer during the subsequent days according to drug availability.

   As a consequence, it is expected that (a) patients registered in the study cohorts will receive the drug at a variable time distance from the date of registration,due to drug availability, and (b) some registered patients might not receive the drug at all, due to hospital discharge or death before being treated.

Such considerationsprompted this amendment to better clarify how inclusion criteria are declined, and how the analysis populationsare to be defined to pursue the primary study end-point. In addition, a new opportunity for statistical analysis arises in the overall population of patients registered prospectively before receiving the drug (phase 2 and observational prospective cohort). Finally, the removal of an exclusion criterium has also been proposed within the Scientific Committee.

Amendment2, 20 April, 2020

Overall Rationale for the Amendment:

The amendment 2 leading to the version 3 of the protocol is based on the availability of new and more detailed information on the outcome of the disease in Italy and on the need of dealing with the rate of missing data in the study, as explained below. The proposed modifications of the protocol have been discussed within the Independent Data Monitoring Committee (IDMC) who agreed on them and formulated specific recommendations.

1. Biweekly report of ISS on deceased COVID-19 patients report median hospitalization times equal to 10 days and 5 days from onset of symptoms and death, and hospitalization and death, respectively. Accordingly, it appears that delaying death assessment at 1 month may be redundant and death estimate at 14 days might be very informative, being also less prone to possible loss of information.Therefore, we proposed to add the 14-days death rate as co-primary endpoint together with the 1-month death rate. As a consequence, the alpha error was splitted and will be 0.025 for each end-point.
2. With the availability of more Italian data, it is evident that the initial null hypothesis was seriously underestimated and should be refined. However, a main concern is the accessibility of data referring to patients hospitalized with severe or critical COVID 19 pneumonia. Thanks to the Italian National Institute of Health (ISS) we accessed to confidential data referring to Veneto region,as for April 15, that showed death rates of 15.6% (day 14) and 28.2% (day 30)calculated by the Kaplan-Meier product limit method. According to data reported by Minister of Health as of the same day, these estimates are not simply applicable to the whole Italian population, because Veneto is among the regions with lowerdeath rate.Therefore, we calculated the ratio of death rate of each region toward Veneto as reference. According to this approach, the overall expected death rate in the Italian population should be equal to 21.7% and 39.2% at 14 and 30 days, respectively. Therefore, we amend the null hypotheses of the phase 2 study (P0) setting the new values of death rates to 20% and 35% at 14 and 30 days, respectively, as suggested by IDMC to account for variability of the baseline estimates.
3. The enrolment of the planned number of patients forthe phase 2 study was completed very rapidly, in less than 24 hours, from March 19 to March 20. But, due to limited availability of the experimental drug, a significant rate of patients did not receive the drug at all or was treated with variable delay after registration. Nevertheless, the IDMC strongly supported the phase 2 study be analyzed according to intention-to-treatment (ITT). Therefore, the primary analysis was turned back to the ITT phase 2 population, as it was in protocol 1.3. However, a further analysis has been introducedto measure death rate according to treatment received (early after registration, late after registration, not received).
4. We anticipate a relevant phenomenon of missing data, due to the fact that registration in the study was instrumental to obtain availability of the experimental drug in a very critical moment of the spread of the disease, and there were many difficulties in data collection within the emergency setting. In addition, drug shortage did not motivate participating institutions to data collection. Therefore, the primary ITT analysis of the

phase 2 study will be probably conducted with a power lower than planned due to missing data. However, to validate and hopefully consolidate the findings of the phase 2 study, the same analyses will be performed in the cohort of patients,consecutively andprospectively registered from March 20 to March 24, 2020who shared the same eligibility criteria of the phase 2 cohort, but could not be enrolled because they exceeded the planned phase 2 study size.

Table of Contents

1. Synopsis

Protocol Title:" Multicenter study on the efficacy and tolerability of tocilizumab in the treatment of patients with COVID-19 pneumonia"

Short Title:Tocilizumab in COVID-19 pneumonia

Rationale

Pneumonia is the most frequent and serious complication of COVID-19, a disease that results from SARS-CoV-2 infection. In particular, SARS-CoV-2 infection induces an excessive and aberrant host immune response that is associated with an acute respiratory distress syndrome, with typical radiological findings and, in most critical patients, with a so-called "cytokine storm", characterized by the plasma increase of many cytokines that produce long-term damage and fibrosis of lung tissue.

Interleukin 6 (IL-6) is a pleiotropic proinflammatory multifunctional cytokine produced by a variety of cell types. IL-6 is involved in various physiological processes such as activation of T-cells, induction of acute phase proteins, stimulation of growth and differentiation of hematopoietic precursor cells, hepatic, cutaneous and neural cell proliferation, metabolism bone, lipid metabolism, and tissue fibrosis. Elevated tissue and serum levels of IL-6 are implicated in the pathogenesis of various inflammatory and autoimmune disorders including many forms of rheumatic diseases; they are also implicated in the cytokine release syndrome (CRS). Tocilizumab is a recombinant humanized monoclonal antibody, of the IgG1 class, directed against both the soluble IL-6 receptor (sIL-6R) and the receptor bound to the membrane (mIL-6R).

Tocilizumab is indicated for the treatment of severe rheumatoid arthritis, systemic juvenile idiopathic arthritis, juvenile idiopathic polyarthritis and for the treatment of the severe or life-threatening cytokine release syndrome (CRS)induced by the chimeric antigen receptor T-cell (CAR-T) in adults and pediatric patients 2 years of age or older.In an experience disclosed by Chinese researchers (Xiaoling Xu1, Mingfeng Han, Tiantian Li et al. Effective Treatment of Severe COVID-19 Patients with Tocilizumab. ChinaXiv: 202003.00026v1) 21 patients with severe or critical COVID-19pneumonia were treated with tocilizumab 400 mg iv (i.e. the expected dose for the treatment of CRS) with reduction of oxygen requirement (15/20), resolution of CT lesions (19/21), normalization of lymphocyte count (10/19), reduction of C-reactive protein levels (16/19), hospital discharge (19/21) with an average hospitalization duration of 13.5 days. These results are considered by the Chinese authors to be very positive and gave rise to the design of a randomized trial (tocilizumab vs control) which will include approximately 190 patients and is expected to reach the planned accrual by mid-May 2020.

Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the efficacy of tocilizumab by describing: o Lethality rate two weeks after registration in the phase 2 ITT population o Lethalityrate one month after registration in thephase 2 ITT population	• Death rate two weeks after registration • Death rate one month after registration
Secondary	
• To evaluatelethalityrate at two weeks and one month according to delay of treatment(received early after registration, received late, not received at all) • To evaluatelethalityrate at two weeks and one month in the subgroup of phase 2 patients who did actually receive the experimental drug (modified ITT) • To evaluatelethalityrate at two weeks and one month in the prospective ITT cohort, with the same eligibility criteria of phase 2, enrolled consecutively after phase 2 closure up to March 24, 2020. • To evaluatein the two cohorts: o Time to death o Respiratory function in terms of: ▪ time to invasive mechanical ventilation (if not previously initiated) ▪ time to definitive extubation (if previously intubated) ▪ time to independence from non-invasive mechanical ventilation ▪ time to independence from oxygen therapy o Whether IL-6 and CRP levelsare predictive of treatment efficacy	• Death rate two weeks after registration • Death rate one month after registration • Date of death • date of intubation (if not previously intubated) • date of definitive extubation (if previously intubated) • date of independence from non-invasive mechanical ventilation • date of independence from oxygen therapy • IL-6 levels • CRP levels

o Trend of the PaO2/FiO2 ratio o Trend of body temperatureand lymphocyte count o Change of the "Sequential Organ Failure Assessment" (SOFA) o Duration of hospitalization o Radiological response • To describe the toxicity of tocilizumab • To describe in the prospective and retrospective population all the endpoints proposed for the phase 2 study	• PaO2/FiO2 ratio • Body temperature • Lymphocyte count • SOFA score • Days of hospitalization • Radiological response • Rate of adverse events codified by Common Terminology Criteria for Adverse Events (CTCAE) v. 5.0

Overall Design

This study project includes a single-arm phase 2 study and a further parallel cohort, enrolling patients with COVID-19 pneumonia.

Phase 2 study

This is a multicenter, single-arm, open-label, phase 2 study. All the patients enrolled are treated with tocilizumab. Two-week (14 days) and one-month (30 days) lethalityratesare the co-primary endpoints.From available data, it can be assumed that two-weekand 1-month lethality ratesfor the population defined by the selection criteria is around 20% and 35%, respectively (P_0). To verify the hypothesis that the experimental drug may produce a 10% reductionof the lethality(from 20% to 10% at two weeks and from 35% to 25% at one month from registration in the study, P_1), 330patients will provide 99% and 95% power, respectively, with a 2.5%bilateral alpha error for each test.The phase 2 population will be defined according to the intention-to-treatment strategy. At least 400 patients will be considered to allow for possible ineligibility found after registration.

Parallelcohort study

Theparallelcohort will include patients who are treated with tocilizumaband cannot enterthe phase 2 study because:

(a) emergency conditions or infrastructural or operational limits prevented registration before the administration of the experimental drug or

(b) they had been intubated more than 24 hours before registration or

(c) the phase 2 study has been closed due to reached sample size.

This means that, after closure of the phase 2 enrolment, patients who might be eligible for the phase 2 study will be included in avalidation cohort study.

The same information planned for the phase 2 cohort is in principle required also for the parallelcohort study, whose sample size is not defined a priori, and that will close at the end of the overall project. All the patients enrolled are treated with tocilizumab.

Treatment and Duration

In both study groups (phase 2 and parallel cohort), participantsreceive one dose of Tocilizumab 8 mg/kg (up to a maximum of 800mg per dose). A second administration (same dose) can be given after 12 hours if respiratory function has not recovered,at discretion of the Investigator.

2. Schedule of assessments

Procedure	**Baseline** before firsttocilizumab administration (possibly no more than 1 day before)	**Treatment and hospitalization period**		**Discharge**	**Follow-up** On day 30
		Before the eventual second administration of tocilizumab	**Every day while hospitalized**		
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	X	
Respiratory assistance assessment	X	X	X	X	
Laboratory assessments[2]	X	X	X	X	
IL-6 (recommended but not mandatory)and CRP levels	X	X	X	X	
12-lead ECG	X	X	X	X	
Vital signs	X	X	X	X	
SOFA score[3]	X	X	X	X	
Thoracic CT scan or Chest XR[4]	X			X	

Procedure	**Baseline** **before firsttocilizumab administration (possibly no more than 1 day before)**	**Treatment and hospitalization period**		**Discharge**	**Follow-up** **On day 30**
		Before the eventual second administration of tocilizumab	**Every day while hospitalized**		
AE review	X	X	X	X	X
Concomitant medication review	X	X	X	X	
Survival follow-up				X	X

[1]twice in a day

[2]At least blood count, bilirubin, AST, ALT, creatinine, PT, PTT, LDH, D-dimer

[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. If baseline evaluation (CT or XR) is available a re-evaluation is planned on day 7 and subsequently if clinically indicated. Anonymized CT or chest XR report will be uploaded in the web-based case report form.

3. Introduction

Tocilizumab is a recombinant humanized monoclonal antibody, of the IgG1 class, directed against both the soluble IL-6 receptor (sIL-6R) and the receptor bound to the membrane (mIL-6R).
Tocilizumab is indicated for the treatment of severe rheumatoid arthritis, systemic juvenile idiopathic arthritis, juvenile idiopathic polyarthritis and for the treatment of the severe or life-threatening cytokine release syndrome (CRS)induced by the chimeric antigen receptor T-cell (CAR-T) in adults and pediatric patients 2 years of age or older.

3.1. Background

3.1.1. SARS-CoV-2induced Pneumonia

Pneumonia is the most frequent and serious complication of COVID-19, a disease that results from SARS-CoV-2 infection. In particular, SARS-CoV-2 infection induces an excessive and aberrant host immune response that is associated with an acute respiratory distress syndrome, with typical radiological findings and, in most critical patients, with a so-called "cytokine storm", characterized by the plasma increase of many cytokines that produce long-term damage and fibrosis of lung tissue.

3.1.2. Interleukin 6

Interleukin 6 (IL-6) is a pleiotropic proinflammatory multifunctional cytokine produced by a variety of cell types. IL-6 is involved in various physiological processes such as activation of T-cells, induction of acute phase proteins, stimulation of growth and differentiation of hematopoietic precursor cells, hepatic, cutaneous and neural cell proliferation, metabolism bone, lipid metabolism, and tissue fibrosis. Elevated tissue and serum levels of IL-6 are implicated in the pathogenesis of various inflammatory and autoimmune disorders including many forms of rheumatic diseases; they are also implicated in the cytokine release syndrome (CRS).

3.1.3. Tocilizumab experience in COVID-19 patients

In an experience disclosed by Chinese researchers (Xiaoling Xu1, Mingfeng Han, Tiantian Li et al. Effective Treatment of Severe COVID-19 Patients with Tocilizumab. ChinaXiv: 202003.00026v1) 21 patients with severe or critical COVID-19pneumonia were treated with tocilizumab 400 mg iv (i.e. the expected dose for the treatment of CRS) with reduction of oxygen requirement (15/20), resolution of CT lesions (19/21), normalization of lymphocyte count (10/19), reduction of C-reactive protein levels (16/19), hospital discharge (19/21) with an average hospitalization duration of 13.5 days. These results were considered by the Chinese authors to be very positive and gave rise to the design of a randomized trial (tocilizumab vs

control), which will include approximately 190 patients and is expected to reach the planned accrual by mid-May 2020.

3.2. Study Rationale

IL-6 might play a key role in the cytokine storm induced bySARS-CoV-2and interfering of IL-6 might be a potentially therapeutic strategy for severe and critical COVID-19.

3.3. Benefit/Risk Assessment

Detailed information about the known and expected benefits and risks and reasonably expected adverse events of tocilizumabmay be found in the Investigator's Brochure of the drug.

However, most of the available information refer to chronic use oftocilizumab at different doses.

In the Chinese experience of tocilizumab400 mg iv once in COVID-19 patients no toxic death and no adverse events were reported.

4. Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the efficacy of tocilizumab by describing: o Lethality rate two weeks after registration in the phase 2 ITT population o Lethality rate one month after registration in the phase 2 ITT population	• Death rate two weeks after registration • Death rate one month after registration
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• To evaluate lethality rate at two weeks and one month according to delay of treatment (received early after registration, received late, not received at all) • To evaluate lethality rate at two weeks and one month in the subgroup of phase 2 patients who did actually receive the experimental drug (modified ITT) • To evaluate lethality rate at two weeks and one month in the prospective ITT cohort, with the same eligibility criteria of phase 2, enrolled consecutively after phase 2 closure up to March 24, 2020. • To evaluate in the two cohorts : o Time to death o Respiratory function in terms of: ▪ time to invasive mechanical ventilation (if not previously initiated) ▪ time to definitive extubation (if previously intubated) ▪ time to independence from non-invasive mechanical ventilation ▪ time to independence from oxygen therapy o Whether IL-6 and CRP levels are	• Death rate two weeks after registration • Death rate one month after registration • Date of death • date of intubation (if not previously intubated) • date of definitive extubation (if previously intubated) • date of independence from non-invasive mechanical ventilation • date of independence from oxygen therapy • IL-6 levels

predictive of treatment efficacy o Trend of the PaO2/FiO2 ratio o Trend of body temperature and lymphocyte count o Change of the "Sequential Organ Failure Assessment" (SOFA) o Duration of hospitalization o Radiological response • To describe the toxicity of tocilizumab • To describe in the prospective and retrospective population all the endpoints proposed for the phase 2 study	• CRP levels • PaO2/FiO2 ratio • Body temperature • Lymphocyte count • SOFA score • Days of hospitalization • Radiological response • Rate of adverse events codified by Common Terminology Criteria for Adverse Events (CTCAE) v. 5.0

5. Study Design

5.1. 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 evidence is 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.

5.2. Overall Design

At its conception, the study project includes a single-arm phase 2 study and a parallel cohort study, enrolling patients with COVID-19 pneumonia.

5.2.1. Phase 2 study

This is a multicenter, single-arm, open-label, phase 2 study. All the patients enrolled are treated with tocilizumab. Two-week (14 days) and one-month (30 days) lethalityrates are the co-primary endpoints.From available data, it can be assumed that two-week and 1-month lethality rates expected in the population defined by the selection criteria is around 20% and 35%, respectively (P_0). To verify the hypothesis that the experimental drug may produce a 10% reduction of mortality (from 20% to 10% at two weeks and from 35% to 25% at one month from registration in the study, P_1), 330patients will provide 99% and 95% power, respectively, with a 2.5%bilateral alpha error for each test. The phase 2 population will be defined according to the intention-to-treatment strategy. At least 400 patients will be considered to allow for possible ineligibility found after registration.

5.2.2. Parallelcohort study

Theparallelcohort includes patients who are treated with tocilizumaband cannot enter the phase 2 study because:

(a) emergencyconditions or infrastructural or operational limits prevented registration before the administration of the experimental drug or

(b) they had been intubated more than 24 hours before registration or

(c) the phase 2 study has been closed due to reached sample size.

This means that, after closure of the phase 2 enrolment, patients who might be eligible for the phase 2 study will be included in the parallelcohort study.

The same information planned for the phase 2 cohort is required also for the parallel cohort study whose sample size is not defined a priori. Patients in the parallel cohort are treated with the same tocilizumab dose and schedule as in phase 2.

5.3. 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.

6. Study Population

6.1. Inclusion Criteria

Participants are eligible to be included in the study if the following criteria apply:

1. Any gender
2. No age limit
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 ≤93%or requiring oxygen therapy or mechanical ventilation either non invasive or invasive (intubated)
7. Patients with criteria #4 and #5 who have been already treated with tocilizumab before registration are eligible for the retrospective part of the parallelcohort.

6.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. Known hypersensitivity to tocilizumab or its excipients
2. Known active infections or other clinical condition that contraindicate tocilizumab and cannot be treated or solved according to the judgement of the clinician
3. ALT / AST> 5 times the upper limit of the normality
4. Neutrophils <500 / mmc
5. Platelets <50.000 / mmc
6. Bowel diverticulitis or perforation

7. Treatments

7.1. TreatmentsAdministered

Study Treatment Name:	Tocilizumab
Dosage formulation:	Tocilizumab20 mg/mL concentrate for solution for infusion
Unit dose strength(s)/Dosage level(s):	Tocilizumab 8 mg/kg (up to a maximum of 800mg per dose). Such dose is the same approved by FDA for the treatment of CRS following CAR-T therapy. A second administration (same dose) can be given after 12 hours if respiratory function has not recovered,at discretion of the Investigator.
Route of Administration	Intravenously
Dosing instructions:	Withdraw a volume of sterile, non-pyrogenic sodium chloride 9 mg/mL (0.9%) solution for injection from a 100 mL infusion bag, equal to the volume of tocilizumab concentrate required for the patient's dose, under aseptic conditions. The required amount of tocilizumab concentrate (0.4 mL/kg) should be withdrawn from the vial and placed in the 100 mLinfusion bag. This should be a final volume of 100 mLinfusion should be performed over a one-hour time.
Packaging and Labeling	Study treatment will be provided in vials. Each will be labeled as required per country requirement of use in clinical practice.
Manufacturer	Roche

7.2. Preparation/Handling/Storage/Accountability

1. The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records). Specific forms for drug accountability will be provided by the promoter.
2. The vial must be stored in a refrigerator (2°C–8°C).Do not freeze. Keep the vial(s)in the outer carton in order to protect from light.

3. Parenteral medicinal products should be inspected visually for particulate matter or discoloration prior to administration. Only solutions which are clear to opalescent, colorless to pale yellow and free of visible particles should be diluted
4. Withdraw a volume of sterile, non-pyrogenic sodium chloride 9 mg/mL (0.9%) solution for injection from a 100 mL infusion bag, equal to the volume of tocilizumab concentrate required for the patient's dose, under aseptic conditions. The required amount of tocilizumab concentrate (0.4 mL/kg) should be withdrawn from the vial and placed in the 100 mL infusion bag. This should be a final volume of 100mL. To mix the solution, gently invert the infusion bag to avoid foaming.
5. After dilution, the prepared solution for infusion is physically and chemically stable in sodium chloride 9 mg/mL(0.9%) solution for injection at 30ºC for 24 hours.
6. From a microbiological point of view, the prepared solution for infusion should be used immediately. If not used immediately, in use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than 24 hours at 2°C–8°C, unless dilution has taken place in controlled and validated aseptic conditions.

7.3. Treatment Compliance

The effective doses of study drugs received by each participant during the study will be recorded together with exact time (date and hour) of administration.

7.4. Concomitant Therapy

There is no contraindication to concomitant treatment (including antiviral drugs) that can be defined in advance given the severity of the disease and the availability of very few data on pharmacological interactions of the tocilizumab schedule planned in this study.

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

8. 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.
- Screening evaluations in the phase 2 and observational prospective cohort must be completed before registration of patients independently of the planned time of drug infusion (depending on drug availability)

8.1. Screening procedures

- Informed Consent Form (see below)
- 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 (if feasible, but strongly recommended)
- Respiratory assistance assessment
- Laboratory assessments: blood count, bilirubin, AST, ALT, creatinine, PT, PTT, LDH, D-dimer (if feasible, but strongly recommended)
- IL-6 and CRP levels (if feasible, but strongly recommended)
- 12-lead ECG (if feasible, but strongly recommended)
- 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

8.2. Treatment and procedures during hospitalization period

- Arterial Blood Gas (ABG) Analysis twice in a day (if feasible)
- Respiratory assistance assessment
- Laboratory assessments: blood count, bilirubin, AST, ALT, creatinine, PT, PTT, LDH, D-dimer
- IL-6 and CRP levels (if feasible)
- 12-lead ECG (if feasible)
- 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 a re-evaluation is planned on day 7 and then if clinically indicated)

- Treatment with tocilizumab 8 mg/kg (up to a maximum of 800mg per dose), after registration, the timing being dependent on drug availability. A second administration (same dose) can be given after 12 hours if respiratory function has not recovered, at discretion of the Investigator.
- AE review (including SAEs)
- Concomitant medication review

8.3. Procedures before discharge

- Arterial Blood Gas (ABG) Analysis (if feasible)
- Respiratory assistance assessment
- Laboratory assessments: blood count, bilirubin, AST, ALT, creatinine, PT, PTT, LDH, D-dimer
- IL-6 and CRP levels (if feasible)
- 12-lead ECG (if feasible)
- 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

8.4. 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)

8.5. Efficacy Assessments

8.5.1. 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. AP/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.

8.5.2. 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.

8.5.3. 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), andscoresfrom 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.

8.6. Adverse Events

8.6.1 Definitions

An **adverse event(AE)** is any untoward medical occurrence in a studyparticipant 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 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.

8.6.2. 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).

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 (+390817702938) 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.

8.6.3. 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.

Term	Description
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.

8.6.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the promoterof 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 promoterwill review all adverse events and issue queries directly to the Investigator reporting the event. The promoterwill 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 tocilizumab/RoActemra (section 6.4.1 as ofthe version 21 released in September 2019) and in the paragraph 4.8 of the Summary of the Product Characteristics of tocilizumab/RoActemra.
- The promoterhas 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 promoterwill report all SUSARs to Eudravigilance through the EVCTM, to all participating Investigators, to all Ethical Committees of participating centres, and to the manufacturer (Roche), 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 promoterwill review and then file it along with the Investigator's Brochure.

- The promoterwill 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.

8.7. Safety Assessments

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

9. Statistical Considerations

9.1. Sample Size Determination

The study is designed as a single-arm single-stage phase 2 study with 2-week and 1-month lethality rate asco-primary endpoints.

Expected 2-week and 1-month lethality rate (P_0): 20% and 35%, respectively.

Auspicated 2-week and 1-month lethality rate (P_1): 10% and 25%, respectively.

Statistical power: 99% and 95%, respectively.

Bilateral alpha error: 0.025% for each comparison.

Sample size needed: 330patients.

At least 400 patients will be considered to allow for possible ineligibility found after registration or missing baseline data.

9.2. Populations for Analyses

For purposes of analysis, the following populations are defined:

Population	Description
Enrolled	All participants who sign the ICF and are registered (including prospective and retrospective cohorts)
Phase 2 - ITT	All patients enrolled in the phase 2 cohort. The ITT population will be the primary analysis set and will provide an estimate of the effect of treatment offer. Because of the limited availability of the study drug immediately after the start of the study it is expected that several patients could have received the drug some days after registration or could not have get it at all owing to death or discharge.

Phase 2 - mITT	The subgroup of patients in the phase 2 ITT population who have received at least one dose of study drug. The mITT population will be the efficacy secondary analysis set and will provide an estimate of the effect of treatment in treated patients.
Validation - ITT	All patients consecutively and prospectively registered after phase 2 closure from March 20 to March 24who were potentiallyeligible for the phase 2 study but could not be enrolled because of the completion of the phase 2 cohort.
Validation – mITT	The subgroup of patients in the Validation ITT population who have received at least one dose of study drug.
Safety	All patients in the phase 2 and validation cohorts who began the infusion of the first dose of study treatment.
Prospective	All patients who have been registered in the parallel cohortbefore receiving the study drug
Retrospective	All patients who have been registered in the parallel cohort after having received the study drug

9.3. 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.).

9.3.1. Efficacy Analyses

Endpoint	**Statistical Analysis Methods**
Primary	**2-weeklethality**is defined as the ratio of the number of subjects dead within 14 days from study start out of phase 2 patients with baseline information. Point estimate will be complemented by exact 97.5% confidence interval. **1-month lethality**is defined as the ratio of the number subjects dead within 30 days from study start out of phase 2 patients with baseline information. Point estimate will be complemented by exact 97.5% confidence interval. In addition, lethality rates will be described separately by age group, and other baseline characteristics of patients.

Secondary	All secondary analyses will be considered as being supportiveof the primary results.

9.3.2. Safety Analyses

All safety analyses will be performed in 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 telephonewith 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 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 promoterand 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 bi-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 are centralized at the Clinical Trials Unit of the National Cancer Institute of Naples and are web-based (http://www.usc-intnapoli.net).

Data collection is electronic through the above website (http://www.usc-intnapoli.net), or by paper CRF transmitted by fax to +39 081-7702938, as soon as possible after completion. For contacts for registration and data collection, see contacts page.

13. Administrative aspects

This is a non-profit investigator initiated trial. In this trial, the experimental drug tocilizumab will be provided at no cost by the manufacturer (Roche).

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.

14. Coordinating centre contacts

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