Cumulative adaptive, multiarm, multistage and multicentre randomized clinical trial with immunotherapy for Moderate COVID-19

(the AMMURAVID trial)

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Study Protocol and Synopsis

Summary

1 BACKGROUND & RATIONALE

1.1 Introduction

SARS-CoV-2 pandemic is causing a worldwide health emergency, due to a 10-20% of infected subjects that develop a severe illness, mainly involving the respiratory system.

The clinical course of severe COVID-19 is characterized by interstitial pneumonia that can rapidly evolve to ARDS and multi-organ failure with hyperferritinhemia, hepatic dysfunction, prolongation of clotting times and mycrothrombosis reminiscent of diffuse intravascular coagulation (1,2). Mild to moderate viral infection does not associate with high elevation of acute-phase reactants, such as C-reactive protein (CRP). However, severe COVID-19 typically shows an abrupt clinical deterioration associated with a high spike in CRP levels. This clinical picture recalls features of septic shock, of cytokine-release syndrome (CRS) after chimeric antigen receptor (CAR)-T cell therapy, and of macrophage activation syndrome (MAS) - a potentially lethal sterile systemic inflammatory condition characterized by a cytokine storm resulting in multi-organ failure (3). MAS typically complicates hyper-acute idiopathic rheumatic conditions such as systemic-onset juvenile idiopathic arthritis (so-JIA), and is typically treated with antagonist of interleukin-1 (IL1) or IL6 (3). Non-idiopathic variant of MAS are known as hemophagocytic lymphohistiocytosis, and are typically triggered by cancers or infections such as Epstein-Barr virus –in presence of concurrent genetic predisposition- or Ebola virus (4).

The pathogenesis of the rapid clinical deterioration in severe COVID-19 is poorly understood. SARS and SARS-CoV-2 are betacoronavirus, characterized by positive-sense single-stranded RNA genome and a surface envelope. SARS virus, which share >80% homology with SARS-CoV-2, has scarce direct cytopathic effect (5). In rats infected with coronavirus neutrophil depletion results on the one hand in higher mortality and on the other hand in reduced lung damage (6). SARS-CoV-2 viral load in the human upper airways does not correlate with clinical severity (7). Therefore, it is possible to hypothesize that innate immunity -which has evolved a protective action during inflammation- may become dysfunctional in specific circumstances such as in the case of significant viral replication, thus contributing to severe organ damage and mortality. The observation of the abrupt clinical deterioration in parallel with the spiking CPR levels as well as the clinical similarities with septic shock and MAS support this contention. However, it is unclear which aspects innate immunity contribute to disease severity during COVID-19 and which other are protective.

1.2 Rationale

The current medical emergency has deeply stressed the entire healthcare chain, from personnel and hospital directly caring for patients to pharma industries, which risk to rabidly run out of stocks of potentially effective drugs. A rapid identification of multiple effective strategies is required, in order to spread the high demand for effective therapies over multiple potential production chains. Thus, we designed a pragmatic randomised trial with a cumulative adaptive multiarm multistage (MAMS) design, to contemporary test multiple immunomodulatory strategies, while minimizing time losses and recruitment of controls (8).

Randomization is a pivotal step toward a better understanding of best therapy against COVID-19 as we do not expect that any of the immunomodulatory agents will have a large effect on the risk of death, but if any had just a moderate effect and was widely practicable then this could avoid large numbers of deaths. Conversely, reliable demonstration that certain agents have no material effect on major outcomes would be of value for avoiding potential toxicity and for optimizing resource allocation.

1.3 Background

Considering that many hyper-inflammatory conditions respond to antagonists of the inflammasome/IL1 axis or of the IL6 axis, these represent interesting candidates. Available pharmacological strategies directly tackle these cytokines and their cognate receptors outside the cells or the intracellular signal transduction

machinery activated by cytokine receptors such Janus kinases (JAKs). In parallel with cytokines and cellular activation, innate immunity relies on complement, which activation can further enhance inflammation and may result in direct cytotoxicity due to C5 activation and the formation of membrane attack complex.

The rationale for each therapeutic strategy is reported below:

IL6 axis. SARS and influenza A virus infections cause rising IL-6 levels (9). In Influenza A virus infection, a severe disease course parallels a highly elevation in IL6 plasma levels (10); a module including IL6 levels was correlated with acute lung injury, shock, requirement of ECMO/death (11). In mice with influenza A, virus is able to shape innate immunity by suppressing intracellular IL6 signalling thus resulting in much higher IL6 levels, inflammation, viral replication, lung damage and mortality (12). In humans with COVID-19, plasma IL6 levels are raised, especially in the subset of patients with more severe clinical phenotype (13). IL6 is a strong inductor of CRP release by the liver and it is reasonable to expect that rising IL6 drives spiking CRP levels upon clinical deterioration. Tocilizumab is a monoclonal antibody that recognizes the IL-6 receptor, and is approved for the treatment of rheumatoid arthritis, juvenile idiopathic arthritis, and cytokine-release syndrome (CRS) following CAR-T cell therapy, where IL6 plays a central role (14,15). In China, about 200 patients with Severe COVID-19 have been treated with tocilizumab with the administration protocol used for CRS (source: Roche Ltd, China). Despite results have not yet being published, the Chinese CDC has approved IV tocilizumab for severe COVID-19 and for those requiring intensive care (www.chinacdc.cn). This approach seems promising and although there is no results published from several trials ongoing on IV tociluzumab, it has been included in the recent documents of the Italian Society of Infectious Diseases, section of Lombardy (16), Veneto (Protocollo terapeutico infezioni da SARS-Cov-2, v 3.0 del 27.3.2020), and Emilia Romagna (Protocollo terapeutico per la terapia antivirale dei pazienti con infezione da COVID19, update del 21 marzo 2020). Preliminary data on siltuximab, a chimeric monoclonal antibody against IL-6, seems also promising. Interestingly following a single-dose administration, the pharmacodynamics of subcutaneous tocilizumab on soluble IL6-Receptor and CRP were comparable to an equal intravenous dose (17), suggesting bioequivalence of the two administration routes. Plasma concentrations peaked from 36 h after sc administration, reaching similar levels to that obtained with iv administration after 2.5 days. Other monoclonal antibody against IL6 receptor, sarilumab is used in its subcutanoues preparation. Considering the simplicity of administration in the emergency setting with potential of shortage of healthcare personnel and resources, the presence of larger worldwide stocks for the subcutaneous preparations as compared to the iv preparation (where available), the similarities in the pharmacodynamics between the two routes of administration, and the moderate disease severity of the patients enrolled in this study, subcutaneous tocilizumab has been selected for this trial.

Inflammasome/IL1 axis. SARS-CoV-2 virus causes pyroptosis, a form of pro-inflammatory programmed cell death associated with cytokine release such as IL1-β (18). Observation in bats further support this contention: various bat species are develop less intense inflammation during viral infections (including infection by MERS virus) as compared to humans or mice, due to an overall dampening of NLRP3 inflammasome activation by multiple mechanisms, both at RNA and protein levels (19). Accordingly, these bats tolerated viral infections with no clinical disease and limited pathology even during the phases of high viral load (19). Importantly, this modulation of inflammation had no impact on the overall viral loads (19). NLRP3 is a sensor protein that upon activation can trigger the assembly of a multimolecular complex named NLRP3 inflammasome, resulting in activation of pro-caspase1, secretion of inflammatory cytokines such as IL1-β and IL18, and potentially pyroptosis. In vivo studies with transgenic mice carrying human MERS virus receptor (18) and observational studies in humans affected by with SARS have shown that betacoronavirus infection results in NLRP3 inflammasome activation, IL1-β and IL6 secretion (9).

It is likely that viral infection induces NLRP3 activation by more than a single mechanism. Viral protein Viroporin 3a of SARS virus has been shown to activate NLRP3 (20). Anaphylotoxin C5a secretion and interaction with C5aR1 is required to activate NLRP3, IL1-β release and trigger pyroptosis in MERS-infected

transgenic mice carrying human virus receptor (18). In general, multiple pattern-recognition receptors (PRRs) can induce NLRP3 activation and pyroptosis, which can become a double-edged sword (21). Indeed, the direct inhibition of NLRP3 with MCC950 results in an improvement in survival and acute lung injury in mice infected with influenza A virus (22), thus representing the proof of concept that dysfunctional inflammasome activation during respiratory virus infection can result in dysfunctional lung damage and lead to death.

In the absence of direct NLRP3 inflammasome inhibitors, the best available strategy is to block the of innate immunity cytokine cascade. Thus, the inhibition of NLRP3 inflammasome products such as IL1-β is a good potential therapeutic target. Canakinumab is a monoclonal antibody that blocks IL1-β. Blocking IL1 signalling by knocking-out IL1-RA in mice resulted in a more severe clinical course and higher mortality after infection with Ebola virus (23). Canakinumab (2-8 mg/kg) is registered for conditions with dysfunctional innate immunity and NLRP3 inflammasome activation such as autoinflammatory diseases (Cryopyrin-Associated Autoinflammatory Syndromes -CAPS-, familial mediterranean fever – FMF, TNF receptor-associated periodic fever syndrome –TRAPS, hyper-IgD syndrome/mevalonate kinase deficiency), gout, Still disease (including adult-onset Still-disease and systemic-onset-juvenile idiopathic arthritis). Moreover, canakinumab is effective for the treatment of MAS, even if the highly inflammatory milieu may require a dose increase (24). Canakinumab and other IL1 antagonists confer a lower risk of infection as compared to other biologics including tocilizumab (25,26). A very large trial showed that canakinumab reduce the risk of recurrent cardiovascular events and without identifying any increased risk of infections (27).

JAK inhibitors. Since the cytokine network is highly redundant, an alternative strategy is to antagonize the intracellular transduction cascade activate by type I/II cytokine receptors. JAK inhibitors have been developed at the purpose. JAK inhibitors antagonize the signalling of more than a single cytokine, depending on their pharmacological properties (28). Baricitinib is a selective JAK1 and JAK2 inhibitor with moderate activity versus tyrosine kinase 2 (TYK2, another member of the JAK family) and minimal activity against JAK3. A short half-life, a good safety profile and a reduced potential for drug interference are important features supporting the use of baricitinib in acute patients. Baricitinib is approved for rheumatoid arthritis. Moreover, artificial intelligence suggested that baricitinib might also inhibit viral entry due to inhibition of AP2-associated protein kinase 1 (AAK1), one of the regulators of endocytosis (29). Baricitinib is orally administered. Following administration, peak plasmatic concentration occurs within 1.5 h, with an elimination half-life of about 8 hrs (30). Accordingly, the plasmatic concentration peak after the first dose is about 70% that occurring at steady state with daily administration.

Steroids: Steroids represent one of the cornerstone of management of hyper-inflammatory syndromes such as MAS or HLH. However, their use in COVID is not routinely performed at the moment. Most studies about the use of corticosteroids in SARS are controversial; one reported arm while another one suggested benefit (31,32). However, most of these studies were observational with potential bias due to the use of corticosteroids in most severely treated patients. The Chinese Thoracic Society published a consensus statement suggesting a careful use of steroids in COVID-19 patients, with a low-to moderate dose (≤0.5-1 mg/kg die) and with an overall duration ≤7 days (32).

2 OBJECTIVES AND OUTCOMES

The aim of this pragmatic trial is to assist the access to experimental intervention against SARS-COV-2 in order to provide reliable information on the actual efficacy on immunomodulatory therapies on the clinical evolution of a diverse set of subjects with COVID-19.

2.1 Primary objectives

The primary objective of this nation-wide randomised trial is to assess whether immunosuppressive agents in addition to hydroxicloroquine can reduce the progression to very severe respiratory failure with PaO2/FiO2 ratio < 200 mmHg (ARDS-range).

2.2 Primary outcomes

Progression to very severe respiratory failure (PaO2/FiO2 <200 mmHg) at day 10

2.3 Secondary objectives

The secondary objectives are to assess any effects of these immunomodulatory drugs on surrogate markers of COVID-19 severity and course with particular attention towards modelling kinetics of markers of immune response associated with disease evolution. Another secondary objective is to verify the safety of the immunomodulatory agents for during COVID-19. Surrogate markers of COVID-19 course will include:

a) Clinical deterioration, defined as at least one of the following:
   - Death
   - Need of orotracheal intubation/ECMO
   - Increase in NEWS-2 score ≥ 2 from baseline
   - Increase in MELD score ≥ 8 from baseline
b) Mortality
c) Need of orotracheal intubation or ECMO
d) Evolution of NEWS-2 and MELD scores
e) Clinical improvement, defined as one of the following
   - Discharge
   - Absent ventilator support, NEWS-2 score ≤3 and MELD ≤13
f) Discharge
g) Defervescence
h) Course of blood tests and PaO2/FiO2

2.3.1 Exploratory objectives (sub-studies)

1) Course/clearance of plasma and sputum virus
2) To verify changes in plasma inflammatory cytokines after immunotherapy
3) To Identify predictive factors of response to immunotherapies

Objectives	OUTCOME
Primary	
• Prevention of very severe respiratory failure at day 10	• Proportion of patients with PaO2/FiO2 <200 mmHg at day 10 in each intervention arm as compared to the control arm
Secondary	
• Prevention of very severe respiratory failure	• Proportion of patients with PaO2/FiO2 <200 mmHg at day 7, 14, 21, 28 in each intervention arm as compared to the control arm • Time to development very severe respiratory failure (PaO2/FiO2 <200 mmHg) in each intervention arm as compared to the control arm
• Prevention of clinical deterioration	• Proportion of patients with clinical deterioration at day 7, 10, 14, 21, 28 of each intervention arm as compared to the control arm

	• Time to clinical deterioration of each intervention arm as compared to the control arm
• Safety of the interventions	• Proportion of number of AEs and SAEs (according to the Common Terminology Criteria for Adverse Events –CTCAE, Version 5.0) of each arm as compared to the control arm at day 7, 10, 14, 21, and 28
• Prevention of mortality	• Proportion of dead patients at day 7, 10, 14, 21, 28 of each intervention arm as compared to the control arm • Survival analysis of each intervention arm as compared to the control arm
• Reduction of the requirements of orotracheal intubation/ECMO	• Proportion of patients requiring orotracheal intubation/ECMO at day 7, 10, 14, 21, 28 of each intervention arm as compared to the control arm • Comparison of the days with orotracheal intubation/ECMO in each interventional arm as compared to the control arm
• Improvements in the NEWS-2 and MELD scores	• Comparison of the course in the NEWS-2 and MELD scores in each investigational arm as compared to the control arm
• Velocity in clinical improvement	• Time to clinical improvement of each intervention arm as compared to the control arm
• Fever disappearance	• Proportion of patients on persistent defervescence (last day of T>37°C, without recurrent T>37.0° for at least 4 days) at day 7, 10, 14, 21, 28 of each interventional arm as compared to the control arm • Comparison of the time to persistent defervescence (last day of T>37°C, without recurrent T>37.0° for at least 4 days) of each interventional arm as compared to the control arm
• Velocity in discharge	• Proportion of patients discharged at day 7, 10, 14, 21, 28 of each interventional arm as compared to the control arm • Time to discharge of each interventional arm as compared to the control arm
• Changes in blood test	• Comparison of the course of blood test in the different arms: ○ FBC ○ Creatinine ○ Bilirubin

	○ Albumin ○ LDH ○ AST/ALT ○ CK ○ CRP ○ IL-6 ○ troponin T ○ ferritin ○ prothrombin-time (INR) ○ lipid profile (triglycerides, HDL-cholesterol, TOTAL-cholesterol) ○ D-dimer ○ PaO2 (arterial gas analysis) and PaO2/FiO2

3 STUDY DESIGN

3.1 Overall design

Cumulative adaptive, multiarm, multistage (MAMS) and multicentre randomized trial (8) with seven interventional arms.

After randomisation, patients will be enrolled in one of the 7 arms for a 7-days intervention period. After the intervention period, all patients will be followed up until day 28 or discharge. In the case of early termination of follow-up due to discharge before Day 28, the local medical investigator will assess the presence of AEs and SAEs at day 28.

The seven arms of intervention include: hydroxicloroquine (HCQ), HCQ + tocilizumab, HCQ + sarilumab, HCQ + siltuximab, HCQ + canakinumab, HCQ + baricitinib, HCQ + methylprednisolone, according to **Figure1**.

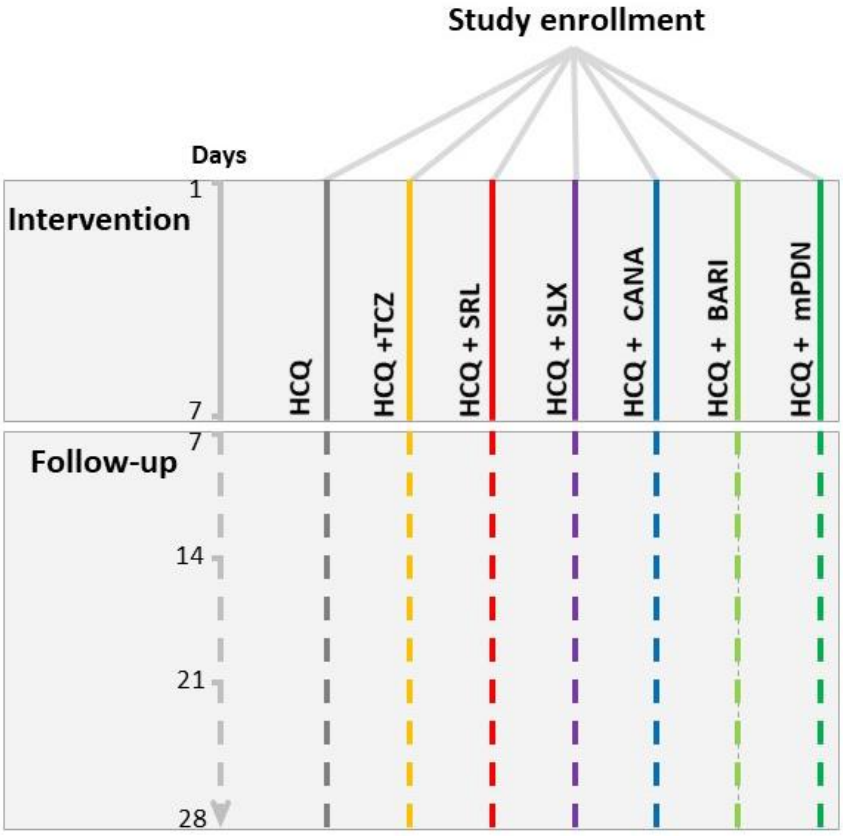

Figure 1: design of the AMMURAVID trial*. HCQ= hydroxicloroquine, TCZ= tocilizumab, SRL= sarilumab, SLX= siltuximab, CANA= canakinumab, BARI= baricitinib, mPDN= methylprednisolone*

4 PARTICIPANT AND STUDY COMPLETION

4.1 End of follow-up definition

A participant is considered to have completed the follow-up if he/she has either:

a) Suffered death
b) Reached day 28
c) Been discharged earlier than day 28 (however, the patient will be contacted by telephone by the local Investigator to verify the absence of AE/SAEs)
d) Withdrawal from the study

4.2 End of study definition

The end of the study is defined as the date of last visit of last participant. An independent drug safety monitory board (DSMB) can decide to stop the study or one or more arms due to efficacy / futility or safety reasons.

4.3 Withdrawal of patients from treatment

A patient should be withdrawn from the study treatment if it is the wish of the patient of it is needed for medical reasons by the local medical Investigator. When a patient is withdrawn, the date of last administration of the investigational drug and the reason for treatment withdrawal should be clearly described in the relevant sections of the electronic case report form (CRF). If treatment withdrawal is due to an adverse effect, the reason should always be stated as 'adverse event' irrespective of whether this was the investigator's or the patient's decision.

The patient will continue follow-up until the end of the study without taking study treatment.

4.4 Withdrawal of patients from the study

In the case of withdrawal from the study, the patient should be examined as soon as possible. Relevant samples should be obtained and all relevant assessments should be completed, preferably according to the schedule for the last visit. Date and reason for the study withdrawal should be clearly described in the electronic CRF.

5 STUDY POPULATION

5.1 Inclusion criteria:

- Adults aged ≥ 18 years able to provide a valid informed consent to the study
- Documented COVID-19 according to local requirements, with pneumonia at imaging (Chest-X ray or CT)
- High inflammation, one of the following:
  - CRP > 6 times UNL
  - D-dimer > 1500 ng/ml
- PaO2/FiO2 250-400 mmHg

5.2 Exclusion criteria:

- Orotracheal intubation or ECMO support
- Active solid / hematologic cancer (including invasive non-melanoma skin cancer)
- Hypersensitivity or contra-indications to one of the investigational agents (including history of deep vein thrombosis / pulmonary thromboembolism or diverticulitis)
- Other active concurrent viral, fungal or bacterial infections (including active tuberculosis, HIV and HCV/HBV infections)
- Pregnancy/breastfeeding
- Incapability to provide a valid informed consent (including age < 18 years old)
- Heart failure with NYHA ≥ 2 or any acute cardiac or vascular event requiring therapy in the previous 12 months
- Chronic renal failure (baseline GFR < 45 ml/min*1.73m^2)
- Liver cirrhosis moderate / severe (Child-Pugh B or C)
- Chronic respiratory failure requiring O2 therapy or ventilation therapy at home
- Blood neutrophils <500/μL, platelet <50000/μL, Hb levels <80 g/
- ALT/AST > 5 times UNL
- Use of any biologic agent or small molecule inhibitor and other investigational drugs in the previous 3 months (or 5 half-lives)
- Use of other immunosuppressive agents in the last 3 months
- Any other condition judged by the local investigator as a contra-indication to eligibility

6 TREATMENTS

6.1 Concomitant therapy

Background therapy

Background therapy is composed of hydroxicloroquine (loading dose of 400 mg x 2 on the first day of therapy), followed by a dose of 200 mg x 2 die. This regimen is shared across all the study arms, in addition to the specific investigational medicinal products. For those patients already on hydroxicloroquine from at least 24 h at the time of randomisation, no loading dose is required.

Concomitant therapy

Concomitant use of any immunosuppressive agents (including investigational medicinal products and systemic steroids) or any other investigational medicinal product evaluated by other clinical trials is not allowed. If any of these therapies are initiated by decision of the Investigator e.g., as rescue therapy due to worsening of the patient's condition, then the patient should be withdrawn from study.

Antimicrobial therapy and prophylaxis as well as local steroids are not limited (including cutaneous or inhalation steroids). Analgesic therapy, transfusion of blood products parenteral nutrition, and general supportive care are always permitted. Concomitant therapy or prophylaxis with low-molecular weight heparin or pentasaccharid is allowed.

Other therapy considered necessary for the patient's welfare may be given at the discretion of the Investigator. All relevant concomitant therapies will be recorded in the eCRF.

6.2 Treatment administered and dose escalation

At day 1 patients will be randomized during a 7-days intervention period in one of the following arms:

1) **Control arm**: HCQ 200 mg x2*
2) **Tocilizumab arm**: HCQ 200 mg x2* + tocilizumab s.c. 8 mg/kg, to be repeated after 12 h
3) **Sarilumab arm**: HCQ 200 mg x2* + sarilumab s.c. 400 mg, to be repeated after 12 h
4) **Siltuximab arm**: HCQ 200 mg x2* + siltuximab 11 mg/kg ev, to be repeated after 12 h
5) **Canakinumab arm**: HCQ 200 mg x2* + canakinumab s.c. 4 mg/Kg, to be repeated after 12 h
6) **Baricitinib arm**: HCQ 200 mg x2* + baricitinib 4 mg die for 7 days.
   For patients aged > 75 years, baricitinib dose is reduced to 2 mg for 7 days.
7) **Methylprednisolone arm:** HCQ 200 mg x2* + iv methylprednisolone 1 mg/kg for 5 days, followed by 0.5 mg/kg die on day 6 and 7.

The intervention period will last for 7 days, after which all patients will continue to be followed and treated according to clinical needs.

*in case that hydroxicloroquine has been started less than 24 h before randomization, use a loading dose of 400 mg x2 on day 1 or -1 of the study.

Table 1: Tocilizumab dose as a function of body weight

Body weight	Tocilizumab dose	N of syringes/pens
≤ 50 kg	324 mg	2
51-70 kg	486 mg	3
71-91 Kg	648 mg	4
≥ 92 Kg	810 mg	5

Table 2: Canakinumab dose as a function of body weight

Body weight	Canakinumab dose	N of syringes/pens
≤ 56 kg	150 mg	1
57-93 kg	300 mg	2
≥ 94 Kg	450 mg	3

6.3 Method of Treatment Assignment

Patients will be randomized through CLOUD-R platform for being equally allocated in one of the 6 study arms. A single pre-defined randomisation list will be developed. Local investigator will be made aware of the treatment allocation upon satisfaction of made Randomization will be performed in blocks of 10 patients per arm.

7 SCHEDULE OF ACTIVITIES (SOA)

7.1 Screening visit

- Assessment of comorbidities
- Quantiferon test. Patients without evidence of active infection can be enrolled while waiting for results. In case of latent tuberculosis, the patient will be offered to undergo antitubercular prophylaxis after the study conclusion
- Pregnancy test (only for women in fertile age)
- HBV/HCV/HIV serology (anti HCV and HCV RNA if positive, antiHIV, HBsAg and anti HBcore). Patients without evidence of active infection can be enrolled while waiting for these results. In case of previous contacts with HBV, patients will undergo antiviral prophylaxis with lamivudine or entecavir to prevent viral re-activation.
- NEWS-2 score
- MELD score
- Reduced biochemistry panel: FBC, CRP, arterial blood gases, fasting glucose, creatinine, bilirubin, AST/ALT, PT-INR
- Chest X ray

7.2 Day 1-Randomisation

- Randomisation
- If screening the screening visit does not occur on day 1:
    - NEWS-2 score
    - MELD score
    - Extended biochemistry panel: FBC with differential, CRP, arterial blood gases, fasting glucose, creatinine, sodium/potassium, AST, ALT, bilirubin, albumin, prothrombin-time (INR), LDH, CK, troponin T, ferritin, D-dimer, triglycerides, HDL-Cholesterol, Total cholesterol, IL6 (if available)*
- Adverse events
- Investigational medicinal product (IMP) administration (potentially to be repeated after 12 h, depending of the allocation arm).

SUBSTUDY:

- Sample for viral assessment in blood (plasma in EDTA, 6 ml tube) and sputum (if available)
- Blood sample for cytokine assessment and genetic analysis (same blood tube as above)

*: lipid profiles, IL6 and samples for viral & cytokine assessment can be collected on day 1 or -1.

7.3 Intervention – Day 2-7

- NEWS-2 score daily
- MELD score: on day 3-5-7
- Adverse events
- Extended biochemistry panel: FBC with differential, CRP, arterial blood gases, fasting glucose, creatinine, sodium/potassium, AST, ALT, bilirubin, albumin, prothrombin-time (INR), LDH, CK,

troponin T, ferritin, D-dimer, triglycerides, HDL-Cholesterol, Total cholesterol, IL6 (if available). To be performed on day 3-5-7*
- Treatment administration (potentially to be repeated after 12 h, depending of the allocation arm).
- IMP administration, depending of the allocation arm.

SUBSTUDY:

- Sample for viral assessment in blood (plasma in EDTA, 6 ml tube) and sputum (if available): day 3, 5, 7*
- Blood sample for cytokine assessment (same blood tube as above): day 3, 5, 7*

*: lipid profiles, IL6 and samples for viral & cytokine assessment can be collected ±1 day apart the due date

7.4 Follow-up

Patients will be followed with daily visits and blood tests up to day ten. Subsequently, patients will receive daily visits and twice a week blood testing according to the following scheme until day 28 or discharge. In the case of discharge before D28, the patient is not required to attend any visit or blood testing, and he/she will be contacted by the local Medical Investigator at day 28, to verify the uneventful clinical course and the occurrence of adverse effects. However, the patient is requested to contact the local Medical investigator in the case of adverse event, in order to verify the need of additional visits or diagnostic tests.

Follow-up – Day 8

- NEWS-2 score
- Adverse events

Follow-up – Day 9

- NEWS-2 score
- Adverse events

Follow-up – Day 10

- NEWS-2 score
- MELD score
- Adverse events
- Extended biochemistry panel: FBC with differential, CRP, arterial blood gases, fasting glucose, creatinine, sodium/potassium, AST, ALT, bilirubin, albumin, prothrombin-time (INR), LDH, CK, troponin T, ferritin, D-dimer, triglycerides, HDL-Cholesterol, Total cholesterol, IL6 (if available)*

SUBSTUDY:

- Sample for viral assessment in blood (plasma in EDTA, 6 ml tube) and sputum (if available)*
- Blood sample for cytokine assessment (same blood tube as above)*

*: lipid profiles, IL6 and samples for viral load & cytokine assessment can be collected ±1 day apart the due date

Follow-up – Day 11

- NEWS-2 score
- Adverse events

Follow-up – Day 12

- NEWS-2 score
- Adverse events

Follow-up – Day 13

- NEWS-2 score

- Adverse events

Follow-up – Day 14

- NEWS-2 score
- MELD score
- Adverse events
- Reduced biochemistry panel: FBC with differential, CRP, arterial blood gases, fasting glucose, creatinine, sodium/potassium, AST, ALT, bilirubin, albumin, prothrombin-time (INR), LDH, CK, troponin T, ferritin, D-dimer, triglycerides, HDL-Cholesterol, Total cholesterol, IL6 (if available)*

*: Blood sampling and MELD assessment during follow-up can be collected ±2 days apart the due date

Follow-up – Day 15

- NEWS-2 score
- Adverse events

Follow-up – Day 16

- NEWS-2 score
- Adverse events

Follow-up – Day 17

- NEWS-2 score
- MELD score
- Adverse events
- Reduced biochemistry panel: FBC, CRP, arterial blood gases, fasting glucose, creatinine, bilirubin, AST/ALT, PT-INR*

*: Blood sampling and MELD assessment during follow-up can be collected ±2 days apart the due date

Follow-up – Day 18

- NEWS-2 score
- Adverse events

Follow-up – Day 19

- NEWS-2 score
- Adverse events

Follow-up – Day 20

- NEWS-2 score
- Adverse events

Follow-up – Day 21

- NEWS-2 score
- MELD score
- Adverse events
- Reduced biochemistry panel: FBC with differential, CRP, arterial blood gases, fasting glucose, creatinine, sodium/potassium, AST, ALT, bilirubin, albumin, prothrombin-time (INR), LDH, CK, troponin T, ferritin, D-dimer, triglycerides, HDL-Cholesterol, Total cholesterol, IL6 (if available)*

*: Blood sampling and MELD assessment during follow-up can be collected ±2 days apart the due date

Follow-up – Day 22

- NEWS-2 score

- Adverse events

Follow-up – Day 23

- NEWS-2 score
- Adverse events

Follow-up – Day 24

- NEWS-2 score
- MELD score
- Adverse events
- Reduced biochemistry panel: FBC, CRP, arterial blood gases, fasting glucose, creatinine, bilirubin, AST/ALT, PT-INR*

*: Blood sampling and MELD assessment during follow-up can be collected ±2 days apart the due date

Follow-up – Day 25

- NEWS-2 score
- Adverse events

Follow-up – Day 26

- NEWS-2 score
- Adverse events

Follow-up – Day 27

- NEWS-2 score
- Adverse events

Follow-up – Day 28

- NEWS-2 score
- MELD score
- Adverse events
- Reduced biochemistry panel: FBC with differential, CRP, arterial blood gases, fasting glucose, creatinine, sodium/potassium, AST, ALT, bilirubin, albumin, prothrombin-time (INR), LDH, CK, troponin T, ferritin, D-dimer, triglycerides, HDL-Cholesterol, Total cholesterol*

*: Blood sampling and MELD assessment during follow-up can be collected ±2 days apart the due date

	Screen-ing	Treatment period					Early Follow-up			Follow-up until discharge‡	End of the study (if early discharge)
Day	**-1/1**	**1**	**2**	**3**	**4-6**	**7**	**8**	**9**	**10**	**11-28**	**28**
Informed consent	X										
Inclusion/exclusion	X										
Demographics and medical history	X										
Chest X Ray	X										
NEWS-2 score	X	X	X	X	X	X			X	X	
MELD	X	X		X	Day 5	X			X	Day 12, 14, 17, 21, 24, 28‡	
AE and SAE	X	X	X	X	X	X	X	X	X	Daily	X
Concomitant medi-cation review	X	X	X	X	X	X	X	X	X	Daily	X
Randomization		X									
IMP administration		X ----------X									
Laboratory											
Pregnancy test	X										
Quantiferon test	X										
HBV / HCV / HIV se-rology	X										
FBC	X	X		X	Day 5	X			X	Day 14, 17, 21, 24,28‡	
Reduced biochemis-try panel#	X									Day 17, 24‡	
Extended biochemis-try panel§		X†		X†	Day 5†	X†			X†	Day 14, 21, 28‡	
Exploratory (6 ml of EDTA-plasma)											
Plasma cytokines		X†		X†		X†			X†		
Blood/sputum for Viral assessment		X†		X†		X†			X†		
Genetic sample		X									

Reduced biochemistry panel#: CRP, arterial blood gases, fasting glucose, creatinine, bilirubin, AST/ALT, PT-INR.

Extended biochemistry panel§: CRP, arterial blood gases, fasting glucose, creatinine, sodium/potassium, AST, ALT, bilirubin, albumin, prothrombin-time (INR), LDH, CK, troponin T, ferritin, D-dimer, triglycerides, HDL-Cholesterol, Total cholesterol, IL6 (if available).

†: lipid profiles and samples for viral load & cytokine assessment can be collected ±1 day apart the due date

‡: Blood sampling and MELD assessment during after day 10 can be collected ±2 days apart the due date

8 STATISTICAL CONSIDERATIONS

8.1 Sample Size Determination

The study will have a MAMS (adaptive multi-arm multi-stage) design with the following features:

a) Open allocation. We decided to conduct an open-label trial, as preparing placebos for the different investigational agents and the seven treatment groups was not feasible in the short time during the current medical emergency.
b) Parallel allocation. Parallel multi arm random allocation is a widely used approach of multiple concurrent comparison of medical interventions. Parallel (i.e. concurrent) allocation between arms is particularly relevant considering the ongoing emergency due to SARS-CoV-2 epidemic, as potential viral mutations in the early phase of the epidemic might be associated to significant variation of the clinical presentation and degree of disease severity
c) Multi-arm. We designed a 6 arm-trial in order to optimize the efforts for randomizing patients against a single control and obtain the best evidence as fastest as possible. All the arms are reasonably set by using adjuvant immunotherapy on top of the standard of care as decided by most recent expert consensus. This design will allow for easily assessing the net effect of each one of the immunomodulators used.
d) Multi-stage (first stage 350 patients and sequential analysis every 50 patients per arms). A multi-stage, sequential design is a pivotal element of the adaptive strategy that allows for assessing participants' data while the trial is ongoing for optimizing trial performance. In this study, we include an N-stage procedure with homogeneous interim analyses each 50 patients enrolled per arm. Sequential design is always associated with inflation of statistical error due to multiple comparisons on the same accrued set of data. However, the large number of patients expected to be enrolled will and the definition of no binding stopping rules will mitigate this issue in the long time.
e) Non-binding stopping rule. Stopping of a specific arm will be decide by the Data and Safety Monitoring Board. We will set no binding stopping rule. However, we define a set of principle to be considered in order to stop an arm or even the entire study:
    1) Significance of the effect against the control arm ($p<0.025$)
    2) Magnitude of the effect, RD 95%CI do not include 10%
    3) Homogeneity of the effect, I-squared across the centres less than 20%
    4) Consistency of results: at least one of the secondary outcome is confirm the effect on primary outcome
f) No sample size pre-calculation. No specific sample size is specified in this pragmatic emergency core protocol. Interim results will be kept under review by an independent Data and Safety Monitoring Board. However, to provide consistency to our study we will carry out an analysis every 50 patients enrolled per arm. It is anticipated that at least several thousand patients will be recruited into the study. The larger the numbers entered the more accurate the results will be, but the numbers that can be entered will depend critically on how large the epidemic becomes. If substantial numbers of patients are hospitalised in the participating centres then it may be possible to enter thousands hospitalised patients with moderate and severe diseases, but realistic, appropriate sample sizes could not be estimated at the start of the trial. Another reason for entering large numbers is that the response to certain treatments may differ substantially between different populations or sub-populations (eg, patients with particular prior conditions, older adults, patients in one or another large country). If sufficient numbers are randomised, it may be possible to obtain statistically reliable treatment comparisons within each of several different countries or types of patient. In the case of difficulties in recruiting an adequate patient number, the scientific committee will evaluate to extend the study group to international centres already collaborating in research networks with the PIs and the scientific committee.

8.2 Statistical Analyses

Statistical analyses relate outcome to the randomly allocated treatment (ie, intent-to-treat).

Primary analysis

All analysis on the primary outcome will be carried out by taking into account the potential effect of each individual component of the adaptive design. The analysis will be carried out by Fisher test combination adjusted for False Discovery Rate for multiple comparisons. Measure of association will be provided according to Risk difference with relative 95% CI and p-value.

Secondary analysis

Binary variables will be modelled according to separate mixed logistic regression models (random intercept at clinical center level) to assess the potential effect of each different treatment arm. All models will be adjusted for the effect of age and gender and potential heterogeneity across the center.

The analysis of repeated quantitative variables (i.e. biomarkers) will be carried out according to tow two-level mixed regression model with random intercept at clinical center (level-2), random intercept at patients (level-1) level and random slope at time level. For all of these variables a kinetic curve will be produced adjusting for age, gender, arm allocation, and virologic status of the patient.

Changes from baseline NEWS-2 and MELD scores, PaO2/FiO2 and blood tests will be modelled by a mixed-effect linear model, performed by using analysis of covariance (ANCOVA) including the treatment arm as a fixed factor and baseline values as a covariate. Least square mean change per arm will be presented with 95% two side confidence intervals.

Post hoc analysis

Finally, we will decide the opportunity to define a set of explorative post hoc analysis by using more complex models. In particular, when the sample size has reached an adequate dimension, we will decide to build a set of mixed regression models to assess potential heterogeneity of results across the following variables: age, sex, presence of diabetes, arterial hypertension, asthma, COPD, enrolment before or after the firth day from symptoms onset, concomitant therapy (eg low-molecular weight heparin), geographical areas, and/or model kinetic of specific biomarkers (latent growth curve) that may predict response to therapy. All these analysis, that are observational in nature, will be decided according to primary results on pre-planned analysis and on the advice of the DSMB.

9 ETHICAL DISCLOSURE

The study will be performed in accordance with the GCP, the Helsinki declaration and national laws. Due to safety requirements due to biological hazard the patient may be not be asked to sign any written consent form. In this case, informed consent will be obtained verbally in the presence of a physicians and witnessed by another healthcare professional (the Physician and the healthcare professional will be asked to sign the consent form). The patient is free to abandon the study or withdraw consent in every moment.

10 PUBLICATION POLICY

The PIs are responsible for the final publication of data. Authors must satisfy all of the following ICMJE authorship criteria: 1. Substantial contributions to conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND 2. Drafting the work or revising it critically for important intellectual content; AND 3. Final approval of the version to be published; AND 4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any

part of the work are appropriately investigated and resolved. Data collection, general supervision of the research group, or overseeing the conduct of the study alone does not justify authorship. Publications will be planned by the PI and the scientific and statistical committees. Selection of authors to be involved in the writing process and objectives of the publication must be approved by the PIs prior to the start of the publication. The position of the authors' names in the publication will be based on the ICMJE criteria and discussed with the PIs and the committees. Publication of partial or local data must be approved by the PIs.

11 ABBREVIATION LIST

AE:	adverse event
ALT:	alanine transaminase
AST:	aspartate transaminase
CI:	confidence interval
CK:	creatine kinase
COVID-19:	coronavirus disease 2019
CRF:	case report form
CRP:	C-reactive protein
CRS:	cytokine release syndrome
CT:	computed tomography
BSMB:	drug safety monitory board
ECMO:	extra corporeal membrane oxygenation
EDTA:	ethylendiaminetetraacetic acid
FBC:	full blood count
GCP:	good clinical practice
GFR:	glomerular filtration rate
HCQ:	hydroxychloroquine
HDL:	high-density lipoprotein
ICMJE:	international committee of medical journal editors
IL:	interleukin
INR:	international normalized ratio
IMP:	investigational medicinal product
LDH:	lactate dehydrogenase
NEWS-2:	national early warning score-2
NYHA:	New York heart association
MAMS:	multi-arm multi-stage
MAS:	macrophage activation syndrome
MELD:	model for end-stage liver disease
PI:	principal investigator
SARS:	severe acute respiratory syndrome
SARS-CoV-2:	severe acute respiratory syndrome coronavirus-2
SAE:	severe adverse event
ULN:	upper limit of normal

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PI Signature