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

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Protocol Amendment Summary of Changes Table

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DOCUMENT HISTORY	
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Amendment N 1 (Version 4.5)	22 May 2020
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Study Protocol

Summary

1 ABBREVIATION LIST

AE:	adverse event
ALT:	alanine transaminase
AST:	aspartate transaminase
BARI	baricitinib
CANA:	canakinumab
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
DSMB:	data and safety monitory board
ECMO:	extra corporeal membrane oxygenation
eCRF:	electronic case report form
EDTA:	ethylendiaminetetraacetic acid
FBC:	full blood count
GCP:	good clinical practice
GFR:	glomerular filtration rate
RDV:	hydroxychloroquine
HDL:	high-density lipoprotein
ICMJE:	international committee of medical journal editors
IL:	interleukin
INR:	international normalized ratio
IMP:	investigational medicinal product
Iv:	intravenous
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
MERS:	middle-east severe acute respiratory syndrome

mPDN methylprednisolone

PI: principal investigator

PCR: polymerase chain reaction

PK: pharmacokinetic

RDV: remdesivir

SARS: severe acute respiratory syndrome

SARS-CoV-2: severe acute respiratory syndrome coronavirus-2

SAE: serious adverse event

SAESI: serious adverse events of special interest

SAR: serious adverse reaction

SUSAR: Suspected unexplained serious adverse reaction

TCZ: tocilizumab

ULN: upper limit of normal

SLX: siltuximab

2 BACKGROUND & RATIONALE

2.1 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.

2.2 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. So far, remdesivir is the only agent with encouraging evidence about a potential role for COVID-19. Remdesivir is a nucleotide analogue active, in vitro and in vivo, on SARS-CoV and MERS-CoV by inhibiting the activity of RNA-dependent RNA polymerase (RdRp). In a compassionate-use study clinical improvement was observed in 36 of 53 patients (68%) (8). Two randomized clinical trial have been completed. The first randomised, double-blind, placebo-controlled, multicentre trial including 233 patients did not show statistically significant clinical benefits (9). The second randomized clinical trial (preliminary data) led by the National Institutes of Health's National Institute of Allergy and Infectious Diseases involving 1063 patients found that remdesivir performed better on the primary endpoint of 'time to recovery' when compared to placebo. Results showed a 31% faster time to recovery in patients treated with remdesivir versus placebo. The median time to recovery was 11 days versus 15 days, respectively while mortality was 8% versus 11.6% (respectively). The role of immunotherapies in combination with antiviral agents such as remdesivir remains to be proven. 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 (10).

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.

2.3 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).

The rationale for each therapeutic strategy is reported below:

IL6 axis. SARS and influenza A virus infections cause rising IL-6 levels (11). In Influenza A virus infection, a severe disease course parallels a highly elevation in IL6 plasma levels (12); a module including IL6 levels was correlated with acute lung injury, shock, requirement of ECMO/death (13). 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 (14). In humans with COVID-19, plasma IL6 levels are raised, especially in the subset of patients with more severe clinical phenotype (15). 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 (16,17). 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 (18), 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.

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 (19). 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 (20). Accordingly, these bats tolerated viral infections with no clinical disease and limited pathology even during the phases of high viral load (20). Importantly, this modulation of inflammation had no impact on the overall viral loads (20). 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 (19) and observational studies in humans affected by with SARS have shown that betacoronavirus infection results in NLRP3 inflammasome activation, IL1-β and IL6 secretion (11).

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 (21). 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 (19). In general, multiple pattern-recognition receptors (PRRs) can induce NLRP3 activation and pyroptosis, which can become a double-edged sword (22). 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 (23), 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 (24). 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 (25). Canakinumab and other IL1 antagonists confer a lower risk of infection as compared to other biologics including tocilizumab (26,27). A very large trial showed that canakinumab reduce the risk of recurrent cardiovascular events and without identifying any increased risk of infections (28).

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 (29). 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 (30). Baricitinib is orally administered. Following administration, peak plasmatic concentration occurs within 1.5 h, with an elimination half-life of about 8 hrs (31). 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 (32,33). 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 (33).

3 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 of immunomodulatory therapies in combination with remdesivir on the clinical evolution moderate COVID-19.

3.1 Primary objectives

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

3.2 Primary outcomes

Progression to very severe respiratory failure (PaO2/FiO2 <200 mmHg) or mortality by day 28.

3.3 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) Mortality
b) Progression to very severe respiratory failure (PaO2/FiO2 <200 mmHg)
c) Progression to very severe respiratory failure or mortality
d) Need of orotracheal intubation or ECMO
e) Evolution of NEWS-2 and MELD scores
f) Clinical improvement defined as one of the following
   - Discharge

- Absent ventilator support, NEWS-2 score ≤3 and MELD ≤13

g) Discharge
h) Defervescence
i) Course of blood tests and PaO2/FiO2
j) Development of late complications at 6 months of follow-up

3.3.1 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 changes in PBMCs-related biomarkers after immunotherapy
4) To identify genetic factors predictive of response to immunotherapies
5) To verify pharmacokinetics of the IMPs

Objectives	OUTCOME
	Primary
Prevention of very severe respiratory failure or mortality	• Proportion of patients with PaO2/FiO2 <200 mmHg or dead by day 28 included in each intervention arm as compared to the control arm
	Secondary
Prevention of mortality	• Proportion of dead patients by day 7, 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
Prevention of very severe respiratory failure	• Proportion of patients with PaO2/FiO2 <200 mmHg by 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 very severe respiratory failure or mortality	• Proportion of patients with PaO2/FiO2 <200 mmHg or dead by day 7, 14, 21 in 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 by day 7, 14, 21, and 28
Reduction of the requirements of orotracheal intubation/ECMO	• Proportion of patients requiring orotracheal intubation/ECMO by day 7, 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

Evolution of 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
Velocity in discharge	• Proportion of patients discharged by day 7, 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
Fever disappearance	• Proportion of patients on persistent defervescence (last day of T<37.0°C, without recurrent T>37.0° for at least 4 days) by day 7, 14, 21, 28 of each interventional arm as compared to the control arm • Comparison of the time to persistent defervescence (last day of T<37.0°C, without recurrent T>37.0° for at least 4 days) 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: o FBC o Creatinine o Bilirubin o Albumin o LDH o AST/ALT o CK o CRP o IL-6 o troponin T o ferritin o prothrombin-time (INR) o lipid profile (triglycerides, HDL-cholesterol, TOTAL-cholesterol) o D-dimer o PaO2 (arterial gas analysis) and PaO2/FiO2
Development of late complications at 6 months of follow-up	• Death • Hospital admissions • Proportion of patients developing new medical conditions in each interventional arm as compared to the control arm. Specific focus to: - new-onset interstitial lung disease - new onset respiratory failure requiring O2 therapy or ventilation therapy at home - thromboembolic event

	- ischemic events (stroke, acute coronary syndromes, peripheral ischemias) - arterial hypertension • Proportion of patients with FVC < 70% of predicted, FEV1 < 70% predicted and DLCO < 80% predicted

4 STUDY DESIGN

4.1 Overall design

Cumulative adaptive, multiarm, multistage (MAMS) and multicentre randomized trial (10) with six interventional arms.

After randomisation, patients will be enrolled in one of the six arms for a 10-days intervention period. After the intervention period, all patients will be followed until day 28 or discharge. In the case of discharge before Day 28, the local medical investigator will contact the patients at day 28 by telephone to assess the occurrence of the main outcomes and AEs and SAEs.

At six months after enrolment, the patients will be evaluated to re-assess the occurrence of the outcomes or any other medical conditions, with a specific attention towards interstitial lung disease, respiratory failure, thromboembolic / ischemic events, arterial hypertension.

The six arms of intervention include: remdesivir (RDV), RDV + tocilizumab, RDV + siltuximab, RDV + canakinumab, RDV + baricitinib, RDV + methylprednisolone, according to **Figure1**.

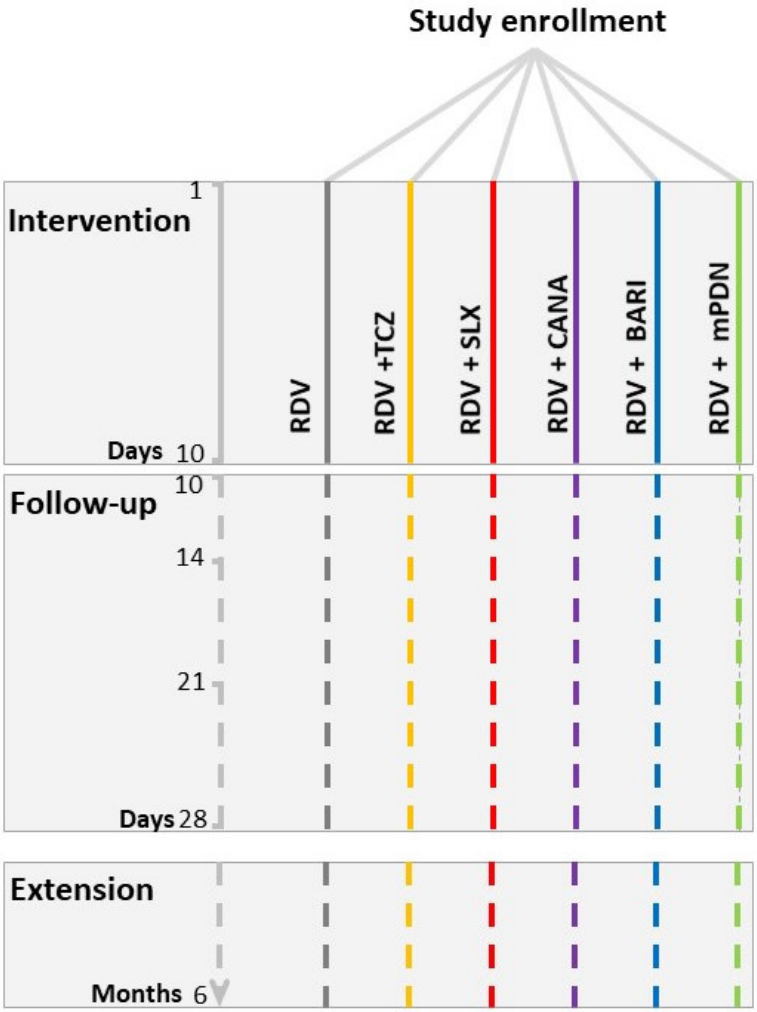

Figure 1: design of the AMMURAVID trial. RDV= remdesivir, TCZ= tocilizumab, SLX= siltuximab, CANA= canakinumab, BARI= baricitinib, mPDN= methylprednisolone

5 PARTICIPANT AND STUDY COMPLETION

5.1 End of follow-up definition

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

a) Suffered death
b) Withdrawal from the study

5.2 End of study definition

The end of the study is defined as the date of last visit of last participant (alternatively, the date of study withdrawal or loss on follow-up of the last participant). An independent data and safety monitory board (DSMB) can decide to stop the study or one or more arms due to efficacy / futility or safety reasons.

5.3 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, including the need to administer rescue therapies. 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. Data on outcomes should still be collected in the eCRF.

5.4 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. In the absence of a specific desire of the patients to be withdrawn from the study, every effort should be made to limit withdrawal solely to study treatment, while continuing to follow the patients and collect data about outcomes and adverse events. As an example, the need to administer rescue therapy due to progression of the underlying disease should be a reason for withdrawal from treatment but not from the study.

6 STUDY POPULATION

6.1 Inclusion criteria:

- Adults aged ≥ 18 years able to provide a valid informed consent to the study
- Documented COVID-19 by direct testing (positive PCR), with lung infiltrates at imaging (Chest-X ray or CT)
- High inflammation, one of the following:
    - CRP > 60 mg/dl
    - D-dimer > 1500 µg/l fibrinogen-equivalent-units (FEU).
- PaO2/FiO2 250-400 mmHg, while in room-air, oxygen therapy or continuous positive airway pressure (C-PAP)
- For women of childbearing potential and men: agreement to use contraception in the case of heterosexual intercourses before day 28 with a failure rate < 1% per year (bilateral tubal ligation, male sterilisation, hormonal contraceptives inhibiting ovulation, hormone-release or copper intrauterine devices. For men enrolled in the study, condom use is allowed.

Rationale for patient selection

The available pathogenic model for COVID-19 suggest that there might be a window of opportunity for immune-targeted therapies, during which severe organ damage has not occurred yet. However, most of subjects with COVID-19 do not develop clinically relevant disease, and it is likely that patients would mostly benefit from immune-targeting therapies are those that will develop severe organ damage due to dysfunctional coronavirus-induce immune activation. Thus we selected patients with mild to moderate respiratory failure and with evidence of significant immune activation as assessed by blood levels of CRP or D-Dimer –which reflect the activation of the coagulation cascade. Evidence-based cut-off values for such variables cannot be defined. The decision on specific cut-off has been defined by consensus based on the clinical experiences of the scientific committee in the treatment of COVID-19 and in agreement with experienced rheumatologists.

With the exception of substudies, clinical laboratory assessment will be provided by local laboratories within each center. Accordingly, local laboratories of the enrolled center are required to use only internationally-accepted certified assays for the clinical variables used, providing relevant certificates to the sponsor. Assays are required to be performed according to international guidelines of the

Clinical Laboratory Standards Institute (CLSI). ISO standards for laboratories (ISO 15189:2007 and ISO/IEC 17025:2005) are strongly recommended. The sponsor will review the assays, the declaration of adherence to international guuidelines and quality certification of laboratories of each center before starting patient recruitment. The sponsor may opt to repeat selected biochemical analysis on the biologic sample centralised to verify that variability of measurements falls within international recommendation by the Clinical Laboratory Standards Institute (CLSI). According to international recommendation, D dimer should be reported in term of FEU (Fibrinogen-Equivalent-Units), to allow standardisation. The C-reactive protein assay required for the study is conventional CRP (high-sensitivity-CRP or cardiac-CRP assays are not required).

6.2 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 diverticulitis or history of deep vein thrombosis / pulmonary thromboembolism within 12 weeks prior to screening)
- 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 O_2 therapy or ventilation therapy at home
- Blood neutrophils <1000/μL, platelet <50000/μL, Hb levels <80 g/l
- 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

7 TREATMENTS

7.1 Concomitant therapy

Background therapy

Background therapy is composed of remdesivir (loading dose of 200 mg on the first day of therapy), followed by a dose of 100 mg die up to day 10. This regimen is shared across all the study arms, in addition to the specific investigational medicinal products. A 10 days-regimen has been preferred considering the concomitant administration of immunosuppressive agents.

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 prophylaxis with low-molecular weight heparin or pentasaccharide is recommended for all patients unless justified.

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.

7.2 Treatment administration

At day 1 patients will be randomized during a 10-days intervention period in one of the following arms:

1) **Control arm**: RDV iv 200 mg on day 1, followed by 100 mg die until day 10
2) **Tocilizumab arm**: RDV IV 200 mg on day 1, followed by 100 mg die until day 10 + tocilizumab IV 8 mg/kg (maximal dose 800 mg), to be repeated after 12 h (range 8-16 hours)
3) **Siltuximab arm**: RDV IV 200 mg on day 1, followed by 100 mg die until day 10 + siltuximab IV 11 mg/kg in single administration
4) **Canakinumab arm**: RDV IV 200 mg on day 1, followed by 100 mg die until day 10 + canakinumab IV 8 mg/Kg in single administration
5) **Baricitinib arm**: RDV IV 200 mg on day 1, followed by 100 mg die until day 10 + baricitinib 4 mg die for 7 days.
   For patients aged > 75 years, baricitinib dose is reduced to 2 mg for 7 days.
6) **Methylprednisolone arm:** RDV IV 200 mg on day 1, followed by 100 mg die until day 10 + 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 10 days, after which all patients will continue to be followed and treated according to clinical needs until discharge.

IV agents will be infused according to the following instructions, summarised in **Tables 1-5**.

Table 1: features of iv drug administration

Agent	Dose	Diluent	Duration of infusion
Remdesivir	200 mg on day1, 100 mg on days 2-10	Saline 0.9% 100 ml	1 hour
Tocilizumab	8 mg/kg (max 800 mg) twice	Saline 0.9% 100 ml	1 hour
Siltuximab	11 mg/kg once	Glucose 5% 250 ml	1 hour
Canakinumab	8 mg/kg once	Glucose 5% 250 ml	1 hour
Methylprednisolone	1 mg/kg on day 1-5, 0.5 mg/kg on day 6-7	Saline 0.9% 250 ml	1 hour

Table 2: Tocilizumab dose as a function of body weight

Body weight	Tocilizumab dose (mg)	Tocilizumab dose (ml)
42	336	16,8
44	352	17,6
46	368	18,4
48	384	19,2
50	400	20
52	416	20,8
54	432	21,6
56	448	22,4
58	464	23,2
60	480	24
62	496	24,8
64	512	25,6
66	528	26,4
68	544	27,2
70	560	28
72	576	28,8
74	592	29,6
76	608	30,4
78	624	31,2
80	640	32
82	656	32,8
84	672	33,6
86	688	34,4
88	704	35,2
90	720	36
92	736	36,8

94	752	37,6
96	768	38,4
98	784	39,2
≥ 100 Kg	800 mg	40

The second tocilizumab administration will be performed 8-16 h after the first administration.

Table 3: Siltuximab dose as a function of body weight

Body weight	**Siltuximab dose**
41-49 kg	500 mg
50-59 kg	600 mg
60-68 Kg	700 mg
69-77 Kg	800 mg
78-86 Kg	900 mg
87-95 Kg	1000 mg
96-104 Kg	1100 mg
≥ 105 Kg	1200 mg

Table 4: Canakinumab dose as a function of body weight

Body weight	**Canakinumab dose**
≤ 47 kg	300 mg
48-65 kg	450 mg
66-84 Kg	600 mg
≥ 85 Kg	750 mg

Table 5: Methylprednisolone dose as a function of body weight

Body weight	**mPDN dose (Days 1-5)**	**mPDN dose (Days 6-7)**
41-44 kg	40 mg	20 mg
45-54 kg	50 mg	25 mg
55-64 kg	60 mg	30 mg

65-74 Kg	70 mg	35 mg
75-84 Kg	80 mg	40 mg
85-94 Kg	90 mg	45 mg
95-104 Kg	100 mg	50 mg
≥ 105 Kg	110 mg	55 mg

7.3 Method of Treatment Assignment

Patients will be randomized through CLOUD-R platform for being equally allocated in one of the 7 study arms. A single pre-defined randomisation list will be developed. Local investigator will be made aware of the treatment allocation upon satisfaction of inclusion and exclusion criteria. Randomization will be performed in blocks of 10 patients per arm.

8 SCHEDULE OF ACTIVITIES (SOA)

8.1 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
- Full blood count with differential
- Arterial blood gases
- Reduced biochemistry panel: CRP, fasting glucose, creatinine, bilirubin, AST/ALT, PT-INR
- Chest X ray

8.2 Day 1-Randomisation

- Randomisation
- If screening the screening visit does not occur on day 1:
    - NEWS-2 score
    - MELD score
    - Full blood count with differential
    - Arterial blood gases
    - Extended biochemistry panel: CRP, 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, 3 ml tube), sputum (if available) and throat/nasal swab*
- Blood sample for cytokine assessment and PBMCs (Plasma in EDTA, 6 ml tube)*
- Blood sample for genetics*
- Serum sample (3 ml tube) for PK assessment*

*: lipid profiles, IL6 and samples for substudies can be collected on day 1 or -1.

8.3 Intervention – Day 2-10

- NEWS-2 score daily
- MELD score: on day 3-5-7-10
- Adverse events
- Full blood count with differential. To be performed on day 3-5-7-10
- Arterial blood gases. To be performed on day 3-5-7-10
- Extended biochemistry panel: CRP, 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 3-5-7-10*
- 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, 3 ml tube), sputum (if available) and throat/nasal swab: day 3, 5, 7-10*
- Plasma sample for cytokine assessment and PBMCs (Plasma in EDTA, 6 ml tube): day 3, 5, 7-10*
- Serum sample (3 ml tube) for PK assessment: day 3, 5, 7-10*

*: lipid profiles, IL6 and samples for substudies can be collected ±1 day apart the due date

8.4 Follow-up

Follow-up will depend on the timing of patient's discharge. Before discharge, the patient will receive daily visits and twice a week blood testing according to the following scheme until day 28. 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. A single visit will be performed at six months, to assess the occurrence of late complications.

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
- Full blood count with differential
- Arterial blood gases
- Reduced biochemistry panel: CRP, 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
- Full blood count with differential
- Arterial blood gases
- Reduced biochemistry panel: CRP, 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
- Full blood count with differential
- Arterial blood gases
- Reduced biochemistry panel: CRP, 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
- Full blood count with differential
- Arterial blood gases
- Reduced biochemistry panel: CRP, 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
- Full blood count with differential
- Arterial blood gases
- Reduced biochemistry panel: CRP, 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

Follow-up – 6 months (assessment can be performed ±14 days apart the due date)

- Medical history (new medical conditions, with a specific attention towards interstitial lung disease, respiratory failure, thromboembolic / ischemic events, arterial hypertension)
- NEWS-2 score
- MELD score
- Adverse events
- Lung function test
- Full blood count with differential
- Extended biochemistry panel: CRP, 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:

- Blood sample for cytokine assessment and PBMCs (Plasma in EDTA, 6 ml tube)

	Screening	**Treatment period**								**Inpatient follow-up**	**Telephone assessment, if early discharge**	**Extended follow-up**
Day	**-1/1**	**1**	**2**	**3**	**4-6**	**7**	**8**	**9**	**10**	**11-28**	**28**	**6 months§**
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	X
Concomitant medication review	X	X	X	X	X	X	X	X	X	Daily	X	X
Randomization		X										
IMP administration		X----------X										
Laboratory												
Pregnancy test	X											
Quantiferon test	X											
HBV / HCV / HIV serology	X											
FBC	X	X		X	Day 5	X			X	Day 14, 17, 21, 24,28‡		X

Arterial blood gases	X	X		X	Day 5	X			X	Day 14, 17, 21, 24,28‡		
Reduced biochemis-try panel#	X									Day 17, 24‡		
Extended biochem-istry panel§		X†		X†	Day 5†	X†			X†	Day 14, 21, 28‡		X
Lung function test												X
Exploratory (6 ml of EDTA-plasma)												
Plasma cytokines		X		X†	Day 5†	X†			X†			X
Cryopreserved BPMCs		X		X†	Day 5†	X†			X†			X
Blood/sputum/swab for viral assessment		X		X†	Day 5†	X†			X†			
Genetic sample		X										
Serum PK		X		X†	Day 5†	X†			X†			

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

Extended biochemistry panel§: CRP, 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 studies, plasma biomarkers, PBMCs and serum PK can be collected ±1 day apart the due date (except for day 1, whose assessment can be performed at day -1 or at day 1)

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

§:assessment and blood analysis/collection can be performed ±10 days apart the due date

9 SAFETY PARAMETERS AND DEFINITIONS

9.1 Adverse event

An adverse event (AE) is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, regardless of causal attribution. Thus, AEs might include:

- 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
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition)
- Recurrence of an intermittent medical condition not present at baseline
- Any deterioration in a laboratory value or other clinical test that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study drug
- Adverse events that are related to a protocol-mandated intervention, including those that occur prior to assignment of study treatment (e.g., screening procedures such as arterial blood gases)

All adverse events occurring during the course of the clinical trial (i.e., from signing the informed consent) will be collected into the eCRF.

The investigator has the obligation to report all AEs occurring during the study and to specify whether any of the AEs result in IMP discontinuation. The investigator also has the responsibility to report AEs occurring within 4 weeks after the last dose of study drug whatever the relationship to this drug. Afterwards, only AE related to study drug and/or study design must be recorded into the eCRF. The investigator should follow each AE until resolution or it is assessed as stable by the investigator.

9.2 Serious adverse event

A serious adverse event (SAE) is any adverse event that meets any of the following criteria:

- Is fatal
- Is life threatening (i.e. the adverse event, in the view of the investigator, places the patient at immediate risk of death)
- Requires or prolongs inpatient hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to study drug
- Is a significant medical event in the investigator's judgment

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an adverse event (e.g., rated as mild, moderate, or severe, according to National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAE], see **Table 2**); the event itself may be of relatively minor medical significance (such as severe headache without any further findings).

Severity and seriousness need to be independently assessed for each adverse event recorded on the eCRF.

Table 1: Adverse Event Severity Grading Scale for Events Not Specifically Listed in NCI CTCAE

Grade	Severity
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; or intervention not indicated
2	Moderate; minimal, local, or non-invasive intervention indicated; or limiting age-appropriate instrumental activities of daily living [a]
3	Severe or medically significant, but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; or limiting self-care activities of daily living [b, c]
4	Life-threatening consequences or urgent intervention indicated [d]
5	Death related to adverse event [d]

NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events.

Note: Based on the most recent version of NCI CTCAE (version 5.0), which can be found at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

[a] Instrumental activities of daily living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

[b] Examples of self-care activities of daily living include bathing, dressing and undressing, feeding oneself, using the toilet, and taking medications, as performed by patients who are not bedridden.

[c] If an event is assessed as a "significant medical event," it must be reported as a serious adverse event.

[d] Grade 4 and 5 events must be reported as serious adverse events

9.3 Serious Adverse events of special interest (SAESI)

The following adverse events have been identified as SAESI 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:

- Serious infections, with the exception of coronavirus infection
- Myocardial infarction, acute coronary syndrome and stroke
- GI perforations
- Stroke
- Serious and/or medically significant bleeding events
- Serious and/or medically significant hepatic events
- Deep vein thrombosis or venous thromboembolism

9.4 Causality assessment

The Investigator must determine the relationship between the administration of the IMP and the occurrence of an AE/SAE as defined below:

Not suspected: An adverse event will be considered related, unless there is evidence that the adverse event has an etiology other than the study drug (e.g., preexisting medical condition, underlying dis-

ease, intercurrent illness, or concomitant medication); and/or the adverse event has no plausible temporal relationship to administration of the study drug (e.g., cancer diagnosed 2 days after first dose of study drug).

Suspected: There is a plausible temporal relationship between the onset of the adverse event and administration of the study drug, and the adverse event cannot be readily explained by the patient's clinical state, concurrent illness, or concomitant therapies; and/or the adverse event follows a known pattern of response to the study drug; and/or the adverse event abates or resolves upon discontinuation of the study drug or dose reduction and, if applicable, reappears upon re-challenge.

9.5 Serious Adverse Reactions (SAR)

SAR are an untoward and unintended response to a study drug at any dose that results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect. SARs requires to be immediately (i.e., no more than 24 hours after learning of the event) reported in the relevant eCRF.

9.6 Suspected Unexpected Serious Adverse Reactions (SUSAR)

SUSARs are Suspected Unexpected Serious Adverse Reactions, meaning that they are SAR for which a reasonable causal relationship with the medicine used is suspected but not confirmed. Unexpected refers to unconsistency with the respective product information. These might include Stevens-Johnson syndrome, anaphylaxis, aplastic anaemia, or anything comparably uncommon.

SUSARs do not include all the events clearly related to progression of the underlying disease (i.e., death due to progressive respiratory failure). SUSARs requires to be immediately (i.e., no more than 24 hours after learning of the event) reported in the relevant eCRF.

9.7 Pregnancy

If a female trial participant becomes pregnant, the subject should be withdrawn from treatments and asked to read and sign consent form to allow the Study Doctor to ask about her pregnancy. Pregnancy has to be reported to the promoter within 24 h from awareness by the investigator. The pregnancy should be followed to determine the outcome, including spontaneous/voluntary termination, details about the birth, maternal/newborn complications, and potential congenital abnormalities. Any SAE experienced during pregnancy must be reported in the eCRF.

9.8 Special situations

Accidental overdose and medication error/misuse (hereafter collectively referred to as "special situations"), are defined as follows:

- Accidental overdose: accidental administration of a drug in a quantity that is higher than the assigned dose
- Medication error: accidental deviation from the protocol in the administration of a drug
- Medication misuse: intentional deviation to the protocol in drug administration

In some cases, a medication error may be intercepted prior to administration of the drug.

All special situations, regardless of whether they result in an adverse event, must be reported on the specific eCRF, by the investigator to the Safety desk immediately (i.e., no more than 24 hours after

learning of the event). Special situations connected with AEs/SAEs as clinical sequelae will be reported as AEs or SAEs at the same time using the eCRF.

9.9 Product complaint

Product complaint is any written or oral information received from a complainant that alleges deficiencies related to Identity, Quality, Safety, Strength, Purity, Reliability, Durability, Effectiveness, or Performance of a product after it has been released and distributed to the commercial market or clinical trial.

Study treatment errors are unintentional errors in prescribing, dispensing, administering or monitoring The investigator must also report all product complaints to the Sponsor. The investigator should document as much information as possible on the IMP Deviation Form, including the product batch number, and forward the form to the Sponsor immediately (i.e., no more than 24 hours after learning of the event) (refer to the pharmacy manual for further details). If the product complaint results in an AE/SAE to the study patient, the event must be reported on the eCRF.

9.10 Expedite reporting of SAESIs and SUSARs

The study will comply with all local regulatory requirements.

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 eCRF, graded according to the corresponding CTCAE term (Version 5.0).

SAESIs, SUSARs, SARs and pregnancy are required to be reported by the investigator on the eCRF immediately (i.e., no more than 24 hours after learning of the event). Pregnancies are not considered AEs.

During the course of the study all the adverse events 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.

9.11 Safety desk

In order to allow an exact regulatory reporting obligation within the required timeframes, the investigator will inform the Safety desk and the CRO of all protocol-defined AESI, SUSARs, SARs and AEs leading to IMP discontinuation and pregnancies by compiling the eCRF within 24 hours.

The Safety desk of the study is represented by the Clinical Research Center (CRC) of the ASST Fatebenefratelli-Sacco, which will be responsible of data assessment for participants' safety and, if necessary, will notify a warning message to the National Competent Authority and inform the .

The Safety Desk and the CRO will review each SAESI, SUSAR, SAR and AE leading to IMP discontinuation and issue queries directly to the Investigator reporting the event. They will be responsible of keeping the DSMB informed and updated as needed. Moreover, the Safety Desk, in capacity of the sponsor, is required to report all SUSARs to the relevant Regulatory Authorities (including the EudraVigilance through the EVCTM), to all participant investigators, to all Ethical Committees of participating centres and to record all AEs according to "Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use ("CT-3")".

9.12 Safety monitoring

An independent Data and Safety Monitoring Board (DSMB) will review SARs, SUSARs, SAESI and AEs resulting in IMP discontinuation every 25 patients enrolled per arm, and will recommend the sponsor about potential safety concerns leading to suspend enrolment in one or more arms due to safety reasons. The frequency of DSMB safety reviews might be increased if necessary.

10 STATISTICAL CONSIDERATIONS

10.1 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 six 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 six arm- trial with a single control in order to optimize the efforts for randomizing patients 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) Three-stages (K=3). 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 assessed the power functions of a three-stage procedure with two interim analyses pre-planned at a sample size of 50, 50 and 100 patients per arm at stages 1-3 (200 patients overall), with Simes intersection test at each stage. The combination test across the stages will be based on the invers normal method. Overall, we will accept a one-sided alpha error of α=0.025.The inflation of statistical error due to multiple comparisons on the same accrued set of data in the sequential design was accounted for according to suitable stopping rules. The group sequential design will be stopping for futility at α_0 probability of 0.5 or efficacy (using O'Brien and Fleming boundaries) with information rates of 0.25, 0.5, 1. No sample size recalculation is forecasted if the sample differences between control and experimental arms is near the futility condition. Sample size recalculation with conditional power up to a maximum of 100 stage-2 patients and 200 stage-3 (300 overall). Conditional power for each subsequent stage is fixed to 80% for a difference between each treatment arm and control of at least 0.15.
   Additional criteria were: 1) Stopping the trial for success criterion if all treatments are shown superior with a difference (drift parameter) between 0 and 0.2.
e) Interim results will be kept under review by an independent Data and Safety Monitoring Board (DSMB).

10.2 Statistical Analyses

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

Primary endpoint 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/Chi-square 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 endpoint analysis

A statistical analysis plan (SAP) will be provided before the database freezing at the first interim analysis.

11 ETHICAL DISCLOSURE

The study will be performed in accordance with the GCP, the Helsinki declaration and national laws. Written informed consent will be obtained for all patients. The patient is free to abandon the study or withdraw consent in every moment.

12 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.

13 SUBSTUDIES

The AMMURAVID trial has optional sub-studies to address exploratory outcomes. These study will investigate:

1) the effect of immunotherapies on viral clearance from blood, sputum and nasal + throat swab.
2) the effect of immunotherapies on plasma and cellular biomarkers in the peripheral blood, including plasma cytokine levels.
3) The presence of predictors of disease progression or adverse events during immunotherapies. This will include genetic biomarkers.
4) Pharmacokinetics of the IMP.

All biologic analyses for the sub-studies will be centralized by the sponsor. Studies are limited to biomarker and genetic research in the field of COVID-19. Sample will be destroyed no later than 15 years after the final Clinical Study Report has been completed.

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