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MINECRAFT Study: MINEralcorticoid receptor antagonism with CanRenone As eFfective Treatment in moderate to severe ARDS in COVID-19, a phase 2 clinical trial.

Trial registration

EudraCT No.: 2021-001360-20

Principal Investigator

Dr. Marco Vicenzi

Protocol version

v1.0, 16/03/2021

Study Type

Phase 2 exploratory randomized clinical trial in open label, monocentric, no-profit

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List of Abbreviations

AE: Adverse Event

AESI: Adverse Event of Special Interest

ACE2: Angiotensin-Converting Enzyme-2

ADR: Adverse Drug Reaction

Ang: Angiotensin

AR: Adverse Reaction

ARDS: Acute Respiratory Distress Syndrome

AT1R: Angiotensin II Receptor Type 1

BID: Bis In Die

CRP: C Reactive Protein

IEC: Independent Ethic Committee

ICF: Informed Consent Form

eCRF: electronic Case Report Form

EDC: Electronic Data Capture

eGFR: estimated Glomerular Filtration Rate

i.v.: intravenous

GCP: Good Clinical Practice

IMP: Investigational Medicinal Product

ITT: Intention To Treat

IWRS: Interactive Web Response Systems

MRA: Mineralcorticoid Receptor Antagonist

PI: Principal Investigator

PRA: Plasma Renin Activity

RAAS: Renin–Angiotensin–Aldosterone System

ReTNIMP: Regardless Trial Non-Investigational Medicinal Product

NSAE: Non Serious Adverse Event

SmPC: Summary of Product Characteristics

SAE: Serious Adverse Event

SARS-CoV-2: Severe Acute Respiratory Syndrome Corona Virus 2

SAP: Statistical Analysis Plan

SAR: Serious Adverse Reaction

SmPC: Summary of Product Characteristics

SUSAR: Suspected Unexpected Serious Adverse Reaction

SOC: Standard of Care

TMPRSS2: Transmembrane Serine Protease 2

ULN: Upper Limit Normal range

Flow-Chart

See Appendix 1

Roles and responsibilities

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Introduction

Background and rationale

Since early December 2019, when the first pneumonia cases of unknown origin were identified in Wuhan, so far almost 116 millions of people across the globe contracted the infection of Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2). Despite the spread of this global pandemic and the volume of clinical trials launched, no consensus in a standardized treatment has been reached, yet.[1, 2] Only oxygen supply, corticosteroids (e.g. dexamethasone), heparin and antibiotics have been indicated as first line treatment to control infection and disease progression.[3]

Once infection is clinically manifested, COVID-19 is primarily characterized by respiratory failure.[4] As previously demonstrated by our research team, cardiovascular reaction to SARS-CoV-2 is appreciable and is linked with gas exchange impairment during disease progression: the increase of blood pressure as well as the lowering of plasma K^+ levels clearly correlates with deterioration of clinical outcomes.[5] Rising of systemic blood pressure may be included in the pathophysiological effects of renin–angiotensin–aldosterone system (RAAS) imbalance and may reflects the whole systemic and pulmonary vascular constriction. Indeed, the foremost hypotheses describing the pathway through which SARS-CoV-2 enters the cells is related to the involvement of Angiotensin-Converting Enzyme-2 (ACE2). As part of the RAAS, ACE2 converts angiotensin II to angiotensin-(1–7) therefore attenuating its vasoconstrictor, pro-fibrotic and pro-inflammatory effects.[4] Moreover, ACE2 serves a counterbalancing role in RAAS, i.e. it cleaves away (i) phenylalanine from angiotensin (Ang) II, converting it to Ang-(1-7), and (ii) leucine from Ang I, converting it to Ang-(1-9). In a paper published in Cell on March 5th 2020, Hoffmann M. et al. describe how the virus uses the viral spike (S) protein to enter the host cell via ACE2 binding.[6] They showed that host cell entry of SARS-CoV-2 depends on a process called S protein priming, which is carried out by cellular proteases such as serine protease transmembrane serine protease 2 (TMPRSS2). This crucial step leads to fusion of viral and cellular membranes. The endocytosis of this complex triggers not only viral entrance into the cell but also ACE2 internalization and sequestration from its active site, the plasma membrane.[4] As a consequence, the downregulation of ACE2 on cell surface prevents this enzyme to exert protective effects in organs, e.g. a cardioprotective effect.[7] Besides coronavirus infections, the potentially deleterious effects of RAAS were reported in heart and lung and medical conditions such as hypertension, heart failure and obesity.[8] COVID-19 leads to disproportionate endothelial damage that disrupts pulmonary vasoregulation, promotes ventilation-perfusion mismatch, and fosters thrombogenesis.[9] Endothelial dysfunction is known to shift the vascular equilibrium towards vasoconstriction thus favoring organ ischaemia, tissue oedema, and a pro-coagulant state.[10]

According to these mechanisms, a progressive activation of the Ang II/AT1R axis along with the final effector aldosterone may be responsible for systemic and pulmonary vasoconstriction, inflammation and oxidative organ damage.[5, 11-13] Accordingly, despite normal values of renin, aldosterone has been found elevated in COVID-19 patients and positively linked to the degree of inflammatory status.[14] This may support the hypothesis that a sort of "primary hyperaldosteronism" is conceivable thus posing the rationale for using mineralcorticoid receptor antagonist (MRA, e.g. canrenone) during acute respiratory distress syndrome (ARDS) induced by SARS-CoV-2 infection. These results are also corroborated by a data-driven

approach published by Ceschi and colleagues demonstrating RAAS inhibitors to exert protective effects in hospitalized COVID-19 patients.[15] The positive effects of MRA are extended to different tissue as endothelium, heart, hippocampus and, in particular, lymphomonocytes.[16] A rise in aldosterone levels generally leads to the development of inflammation, fibrosis and endothelial dysfunction. Indeed, MRA inhibits cardiac and vascular collagen turnover, reduce sympathetic activity, and improve endothelial dysfunction.[11] Recently, few clinical trials aimed at testing the effect of spironolactone, alone or in combination with other molecules, in reducing COVID-19 mortality (NCT04345887; NCT04424134; EudraCT No. 2020-001766-11). Canrenone belongs to the first generation MRA and in Italy it is an approved treatment for primary hyperaldosteronism, secondary hyperaldosteronism-dependent edema and as a second-line treatment for hypertension. According to Cippà and colleagues, also in our hospital setting, the RAAS inhibitor canrenone effectively reduces in-hospital COVID-19 mortality in a retrospective study conducted by our team at the Cardiorespiratory COVID-19 Unit.[12] We can speculate that canrenone may exert some of its positive effects against the SARS-CoV-2 induced ARDS through different mechanisms: it (i) antagonizes aldosterone and spares K^+, which are both altered by COVID-19 infection, (ii) increases plasma levels of circulating ACE2, thus limiting the binding of the pathogen to cellular ACE2, and (iii) exerts a direct anti-inflammatory and antiviral effect that could avoid pulmonary complications related to COVID-19 (discussed in[17-19]).

By antagonizing aldosterone, canrenone might thus interfere with the progression of SARS-CoV-2 infection from the viral phase to the vascular-pulmonary and cytokine fatal phases. Canrenone is the main active metabolite of spironolactone, and offers a panel of advantages compared to its precursor, spironolactone:

- a 20% of spironolactone is not converted to active molecules; thus, the administration of the active metabolite canrenone in moderate-to-severe ARDS may result in a more effective antagonism of excessive aldosterone;

- The use of canrenone, rather than its precursor spironolactone, restrains the potential off-targets effects other undesired, but biologically active, metabolites of spironolactone;

- among the RAAS-inhibiting agents, only canrenone can be administered intravenously triggering a prompt, and potentially therapeutic, biological effect without first pass liver effect;

- canrenone may display a better safety profile than other MRAs: (i) canrenone does not interact with P450 cytocrome, thus reducing the risks of drug-drug interactions; (ii) canrenone in combination with low molecular weight heparins (which are strongly recommended in all COVID-19 hospitalized patients) is less prone to cause severe hyperkalemia.

The aim of this clinical trial is to investigate if canrenone administration as add-on therapy significantly impacts the clinical outcome of hospitalized COVID-19 patients. As comparator, we chose a reference group that receives maximal medical treatment as best available therapeutic and ethical option for these patients. Concomitantly, we will explore the potential molecular mechanisms underlying its pharmacological effect and identify potential biomarkers that, upon specific validation studies, may

represent relevant prognostic as well as predictive biomarkers of efficacy and safety of canrenone administration in COVID-19 patients. Indeed, not only the actors of the RAAS will be dosed and correlated to clinical outcome and canrenone responsiveness, but also a panel of androgens and other structurally related steroids. Androgen involvement in COVID-19 pathophysiology, severity and progression has already been postulated but the correlation between androgen expression and clinical outcome is still debated (reviewed in[20, 21]).

Risk/Benefit Considerations

Standard-of-care treatments for the Reference group will be administrated in accordance with their marketing authorization and authorized SmPC. Thus, no study-specific risks for patients allocated to the Reference Group (Group B) are conceivable but only consequences due to their pathological condition.

MRAs are largely use in the clinical practice for the management of primary hyperaldosteronism, secondary hyperaldosteronism-dependent edema and as a second-line treatment for hypertension. Among them, canrenone is the most manageable and tolerated, with limited adverse events: indeed, only uncommon/rare adverse reactions are reported in SmPC. Conversely to other MRAs, canrenone has a very low affinity to androgen receptor, thus restraining subsequent disturbances of reproductive system. Moreover, in order to minimize the risk of SAR, the eligibility criteria of this trial impose the prior exclusion of patients for whom canrenone administration is contraindicated according to SmPC; adherence to the eligibility criteria is thus essential to ensure that appropriate subjects are selected for participation.

Nevertheless, a canrenone-dependent increase in hematic kalemia is expected and the dosage of the IMP will be adjusted accordingly (Table 1) to mitigate the risk of serious adverse reaction (SAR). Electrolytic balance (Na^+, K^+, Cl^-) is daily monitored in the routine clinical management of these hospitalized patients, thus allowing a precocious detection of unsafe alterations without achieving life-threatening K^+ hematic levels (grade 3 or more in CTCAE v.5, K^+>6.0 mmol/L). Moreover, in the retrospective analysis (Vicenzi, et al., 2020) patients receiving canrenone did not develop severe iperkalemia, with values of hematic K^+ lower than 5.3 mM.

Conversely to spironolactone and eplerenone, canrenone does not interact with P450 cytocrome thus conferring a limited drug-drug interaction and a favourable safety profile, if compared to other MRA; this is particularly relevant if we consider that a large portion of COVID-19 hospitalized patients are old and fragile subjects with comorbidities and assuming politherapies. Of note, the risk or severity of hyperkalemia can be increased when heparin is combined with Spironolactone, but this is not reported for canrenone, indicating that this risk is much lower or negligible. No interaction with the other Regardless Trial Non Investigational Medicinal Products (ReTNIMPs) used in this trial (remdesivir and dexamethasone) has been reported so far, to our knowledge.

With this favourable safety profile, canrenone represents a promising molecule in moderate-to-severe COVID-19 patients. Study findings shall potentially improve COVID-19 patients' management and progression from the viral to the more dreadful inflammatory and respiratory phase of the infection, preventing their admission to Intensive Care Units with limited bed availability. Moreover, this trial may also identify potential

new hematic biomarkers, able to prior define the patient's prognosis as well as predict patients' responsiveness to the therapy. The planned subgroups statistical analyses may also assess the clinical and demographical features of subjects that would benefit the most from canrenone administration.

Therefore, we believe that the expected benefit largely outweighs the minimal risk to subjects.

Objectives

The main aim of the study is to estimate the potential efficacy of i.v. canrenone as add-on therapy on maximal medical treatment versus maximal medical treatment alone in treating moderate-to-severe ARDS due to SARS-CoV-2.

Furthermore, we aim to:

1) investigate whether i.v. canrenone can modulate the COVID-19 progression;
2) evaluate the safety profile of canrenone administration in COVID-19 patients;
3) preliminarily screen potential new biomarkers, prognostic for clinical outcome and/or predictive of efficacy and safety of canrenone;
4) explore the role of RAAS on SARS-CoV-2 infection.

Trial design

Phase II, open label, 1:1 randomized parallel arms, Simon's two stage design, single centre. This phase 2 exploratory trial is designed to preliminarily estimate the potentiality of canrenone in a small number of prospectively enrolled patients at higher risk of progression to hyperinflammatory phase of COVID-19 infection: positive results from this explorative trial may pave the way for a larger, randomized, placebo controlled, double blind clinical trial.

Participants, interventions, and outcomes

Study setting

Teaching and research hospital, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan, Italy.

The hospital has approximately 800 acute care beds and it is a regional reference centre for COVID19 patients since the beginning of the pandemic in late February 2020.

Eligibility criteria

- Inclusion criteria:
    - Age 18 – 80 y.o. Since over eighties are very fragile patients, a lot of confounding unpredictable events may interfere with the trial analyses; thus, these patients will be excluded from this exploratory proof-of-concept trial;
    - COVID-19 diagnosis through swab within 14 days from the beginning of symptoms

- Hospitalization for moderate to severe ARDS (as determined by $PaO_2/FiO_2 \leq 300$ mmHg at admission)
- Serum concentration of potassium ≤4.5 mEq/L
- Consent to participate

- Exclusion criteria:
  - Invasive mechanical ventilation
  - I.v. hydratation with Darrow's solution or half-strenght Darrow's solution underway
  - Acute cardiovascular event (acute myocardial infarction, acute ischaemic stroke)
  - Current malignant disease
  - Creatinine >1.8 mg/dL (for women) and >2.0 mg/dL (for men) or glomerular filtration rate <50 mL/mm
  - Systolic blood pressure <110 mmHg and/or diastolic blood pressure <60 mmHg
  - Known or suspected hypersensitivity to canrenone
  - Hyponatremia
  - Anuria
  - Familial history of porphyria
  - Pregnancy and breastfeeding
  - Known or suspected hypersensitivity to canrenone
  - Inclusion in any other pharmacological clinical trials

- Withdraw criteria:

Subjects may be withdrawn from the study at the discretion of the Investigator or Sponsor due to a safety concern or if judged non-compliant with trial procedures. A subject must be withdrawn from treatment if one of the following applies:

- Subject chooses to withdraw from the study at any time
- Hyperkalemia defined as $[K^+]$hematic > 5.1 mEq/L;
- hyponatriemia defined as $[Na^+]$hematic < 126 mEq/L
- Other intolerable adverse effects
- Major violation of the study protocol
- Other circumstances that would endanger the health of the subject if he/she were to continue his/her participation in the trial

Reasons for withdrawals and discontinuation of any subject from the protocol have to be recorded.

Interventions

Upon signing the Informed Consent, patients will be randomized 1:1 to receive canrenone (Experimental Arm – Group A) or to enter the observational Reference Group (Group B).

Reference Group: maximal medical treatment

Patients randomized to the Reference Group will receive the standard-of-care treatments, according to institutional procedures in force:

- Dexamethasone i.v. 6 mg die for consecutive 5 days
- Methylprednisolone i.v. 40 mg bid for consecutive 10 days
- Low-molecular-weight-heparin i.v. at standardized dose of 70 UI/kg twice
- Remdesivir i.v. 200 mg in bolus (1st day) then 100 mg die for 4 days; remdesivir will be used only in patients supported with low-flow nasal cannula oxygen or Venturi mask
- Antibiotic therapy:
    - azithromycin: 500 mg/die per os for 5 days
    - ceftriaxone: 2 g i.v. die for 8 days

Experimental Group potassium canrenoate (Luvion®) in addition to maximal medical treatment

Patients randomized in the Experimental Group will receive canrenone as add-on therapy to standard-of-care treatments. Different starting doses of i.v. canrenone will be administrated in a single or double infusion per day, for 7 days, according to the serum concentration of potassium at randomization (see Table 1). The 7-day treatment has been chosen since, in our retrospective analysis [12], the improvement of clinical parameters was already appreciable upon 7 days of treatment. Positive effect of canrenone could also be detected as soon as after 48 hrs of treatment.

Table 1

$[K^+]^{HEMATIC}$	I.V. CANRENONE
>4.0 mEq/L	100 mg/24h
≤4.0 mEq/L and >3.5 mEq/L	200 mg/24h
≤3.5 mEq/L	200 mg twice/24h

The dose of i.v. canrenone can be further adapted according to hematic kalemia with referring the Table 1 during the week after randomization.

Canrenone administration will be discontinued in case of intolerable side effects or severe life-threatening hyperkalemia ($[K^+]^{hematic}$> 5.1 mEq/L)/hyponatriemia ($[Na^+]^{hematic}$< 126 mEq/L): canrenone withdrawal will be noted as adverse event due to the treatment (see below the safety endpoints). In this case, data collected till the exclusion of the participant will be analyzed.

No concomitant treatments other than standard-of-care are allowed.

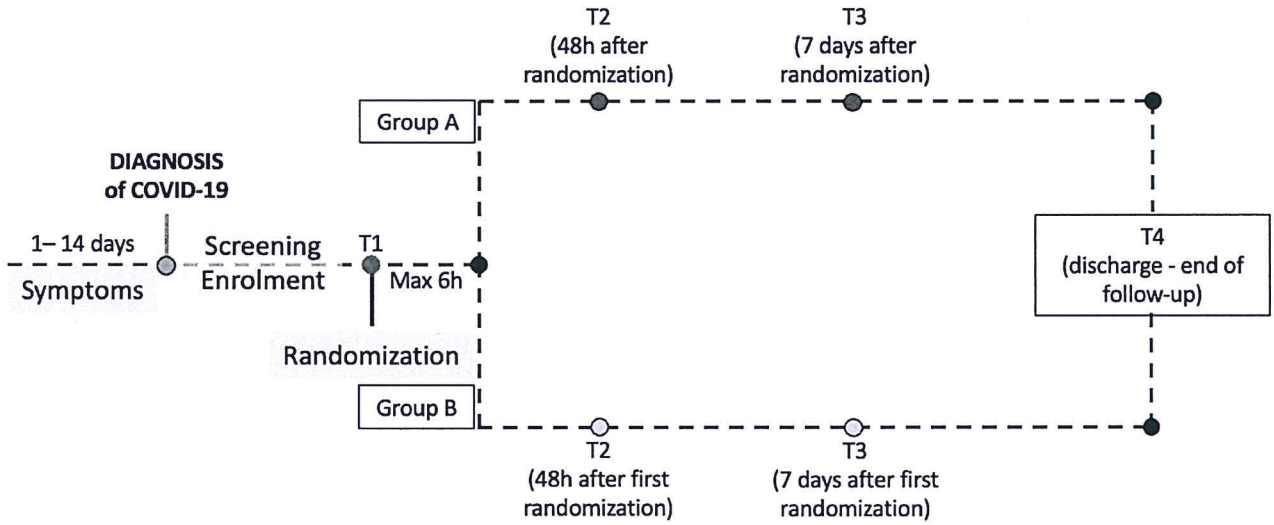
Outcomes

Primary endpoint: in-hospital death. This implies that patients discharged to a long-term care facility will be classified as "discharged alive".

Secondary endpoint:

- Need of invasive mechanical ventilation throughout hospitalization;
- Change in SOFA score from randomization to 7 days after randomization;
- Changes in hemodynamic and respiratory parameters (Heart Rate, Blood Pressure, PaO_2/FiO_2, alveolar-arterial gradient (ΔA-a)), inflammatory status (CRP levels, IL-6, Ddimer and Ferritin) at 48 hours and 168 hours (7^{th} day) from randomization;
- Changes in features of pulmonary interstitial disease assessed by chest X-Ray at 7 days after randomization in both groups;
- Changes in $[K^+]^{hematic}$, renin, AngII, Ang1-7, Ang1-9, aldosterone and structurally related steroids at randomization (T1), and after 48 hrs (T2) and 7 days (T3) and comparison between the two groups of the study;
- Correlation between levels of $[K^+]^{hematic}$, renin, AngII, Ang1-7, Ang1-9, aldosterone and structurally related steroids, at basal level (randomization) and clinical outcomes (in-hospital death, need of invasive mechanical ventilation, SOFA score);
- Duration of hospitalization for alive patients (from randomization to discharge, T4);
- Safety endpoints:
  - Drug intolerance measured as number of AR and SAR as defined in the "Harm" paragraph;
  - Number of hypotensive events defined as systolic blood pressure constantly <90 mmHg and diastolic blood pressure constantly <60 mmHg);
  - Number of Hyperkalemia events defined as $[K^+]^{hematic}$ >5.1 mEq/L;
  - Number of Renal failures defined as eGFR <30 ml/min.

Participant timeline



Group A: i.v. canrenone added on maximal medical treatment
Group B: maximal medical treatment

Sample size

Sample size for group A was calculated according to Simon's two stage design [22] and considering the total hospital mortality occurred in the Cardiorespiratory COVID-19 Unit (mortality risk about 20%) during the first wave of pandemic (from March 2020 to July 2020). We aim at halving hospital mortality (from 20% to 10%) with i.v. canrenone.

The optimal two-stage design to test the null hypothesis that P<=0.80 versus the alternative that P>=0.90 has an expected sample size of 85.52 and a probability of early termination of 0.099. If the drug is actually not effective, there is a 0.038 probability of concluding that it is (the target for this value was 0.050). If the drug is actually effective, there is a 0.196 probability of concluding that it is not (the target for this value was 0.200). After testing the drug on 45 patients in the first stage, the trial will be terminated if only 32 or fewer respond. If the trial goes on to the second stage, a total of 90 patients will be studied. If the total number responding is less than or equal to 78, the drug is rejected.

In parallel, a control group of the same size (n=90) will be enrolled; of note, the control group serves the purpose of 1) equity, ensuring that all patients have an equal chance of receiving either treatment 2) verifying the correctness of the assumption of the null hypothesis, but will not be formally compared.

Total study sample size if the study proceeds to stage 2: 180 subjects.

Sample size calculations were performed with PASS 11 Hintze, J. (2011). NCSS, LLC. Kaysville, Utah, USA. www.ncss.com.

Recruitment

Once hospitalized, patients will be recruited in three selected divisions in order to achieve adequate participant enrolment within the study timeline:

1. Cardiorespiratory COVID-19 Unit (Sacco Building, 1st – 2nd floor)
2. Infectious Disease Unit (Granelli Building, mezzanine floor)
3. Internal Medicine, Hemophilia and Thrombosis Center (Granelli Building, 3rd floor)
4. Internal Medicine, Immunology and Allergology Unit (Granelli Building, 3rd floor)
5. Emergency Medicine Department (Padiglione Guardia Accettazione)

Patients will be screened for inclusion (see eligibility criteria) and then for exclusion. Any of the inclusion criteria may trigger the screening, including consent and data entry in the eCRF (see later).

Study Timeline

Milestone	**Planning**
IEC/CA approvement	May 2021

1st patient inclusion	June 2021
Ad interim analysis (45 patients enrolled in Group A)	February 2022
End of recruiting period	August 2022
Data analysis and final report	December 2022

Assignment of interventions (for controlled trials)

Allocation: Sequence generation

The 1:1 randomization sequence will be produced by permuted blocks of variable size, stratified by division and study stage.

Allocation: concealment

Allocation to treatment will be performed by means of an Interactive Web Response Systems (IWRS) embedded into the REDCap eCRF (see later). A screening/enrolment form will collect data on the eligibility criteria; if all are fulfilled, the patient will be allocated to the study arm in a concealed fashion. Patients will be randomized to the study groups matched for age, sex and Charlson's score (all applicable items according to trial-specific eligibility criteria).

After allocation, the study will proceed in an open label fashion, and patients allocated to the experimental group (Group A) will receive the first dose of the study drug <6 hours from randomization.

After the inclusion of the 45th patient of Group A of the first stage, enrolment will be put on hold until the complete follow-up of the enrolled patients (=discharge of all patients), to verify whether the constraints are met and the study can proceed to the second stage.

Allocation: blinding

Given the study phase (phase 2) and the lack of direct comparative aims, the study will be performed open label.

Data and Biological Samples collection, management, and analysis

Data collection and management methods

Source of the data will be the patient medical chart. We anticipate that some patients' data will be retrieved from clinical records since the majority of study variables are routinely determined. Clinical values relevant for the trial will be recorded on electronic *Case Report Form* (eCRF), specifically designed for this trial (variables and timepoints listed in Appendix 2).

The following clinical data will also be collected, subject to patient's consent, in the COVID-Network registry of the Fondazione. To prevent redundancy, we will design an IT system to avoid double data entry.

Lab-test results required at the time of screening will be collected from the Central Laboratory Digital Repository (Spartito) and accessible from the intranet to the attending physicians. At Basal Visit (T1), 48h and 7d after randomization (T2 and T3, respectively):

- Demographic and general data
- Ventilatory support

All data obtained for this study (see later) will be entered into a local regulation compliant Data Management System, provided by the Clinical Trial Center (CTC) of the IRCCS Ca' Granda Ospedale Maggiore Policlinico Foundation, Milan, Italy. Data will be recorded with an Electronic Data Capture (EDC) system using eCRFs. Specifically, the EDC will be based on the RedCap platform. REDCap is a workflow methodology and software tool that expedites the electronic collection of research data from a single site or multi-site clinical research study. The software supports a secure web-based application for developing fully functional case report forms (CRFs) and surveys. In particular, through REDCap we will implement: (a) Full user authentication (log-on/password) to restrict users to study functions; (b) Real-time data validation, integrity checks for insuring data quality; (c) De-identification options to be applied to data exports to remove fields that contain notes and other information that could identify patient; (d) Centralized, secure storage of research data with back-ups; (e) The study database will be resident on a server in a secure location within the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

- Data will be prospectively revised, by a statistician blind to group allocation, for completeness and congruency, queries will be sent to local investigators every three months.
- Clinical and ventilatory parameters
- Hemogas-analysis
- Routine lab tests

Demographic, clinical and laboratory parameters will be collected at screening (T0), randomization (T1), and after 48 hrs (T2) and 7 days (T3) from randomization by the investigators (see Appendix 2 for details).

In particular:

- Screening variables (T0) include demographic and general data, Charlson's score, ventilatory support, ventilatory and clinical parameters, hemogas-analysis, routine laboratory tests;
- Baseline variables (T1) include ventilatory support, ventilatory and clinical parameters, hemogas-analysis, routine and extra standard-of-care laboratory tests;
- Outcome variables (T2 and T3) include ventilatory support, ventilatory and clinical parameters, hemogas-analysis, routine and extra standard-of-care laboratory tests;
- At T4, discharge from hospitalization will be recorded without further clinical information.

Charlson's score will be calculated at the screening and will be considered as discriminant variable during randomization process. Death will be notified according to clinical management and entered in CRF within

24 hours. At T4, discharge from hospitalization will be recorded without further clinical information. Adverse events will be recorded and entered in CRF according to clinical management.

Database management and quality control for this study are under the responsibility of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan. Essential original records (incl. Institutional Review Board approval, consent forms, CRFs, SAE reports and relevant correspondence) will be retained by the investigator at trial sites as needed to comply with national and international regulation to allow inspection by relevant authorities. The trial database will be maintained for at least 7 years.

Collection and management methods of biological samples

At randomization, T1, T2 and T3, blood sample will be collected to quantify plasma renin activity (PRA), aldosterone, AngII, Ang1-7, Ang1-9. Besides blood collection for routine clinical management, 14 ml supplementary blood volume will be collected using EDTA as an anticoagulant and centrifuged for 15 minutes at 1000g at 2-8°C within 30 minutes. Then supernatants will be stored at ≤20°C in the UOC Cardiologia until batch analysis. Samples will be centrifuged again after thawing before the assays. Repeated freeze-thaw cycles will be avoided; hemolyzed samples are not suitable for testing.

PRA, AngII, Ang1-7quantifications will be performed using the ELISA Kit (purchased by Novus, Bio-Techne) and Ang1-9 concentrations will be measured through the ELISA Kit purchased by Lifespan (via Bio-Techne), according to the datasheets. Analyses will be performed by dott. Leonardo Terranova in the Respiratory Unit and Adult Cystic Fibrosis Center (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan).

Aldosterone quantification will be performed by LC-MS/MS Assay (MassChrom® Steroids, ChromoSystems) according to the user manual. Prof.ssa Fustinoni will be responsible for the analyses that will be performed in the Environmental and Industrial Toxicology Unit (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan). With a single analytical column and one sample preparation, the MassChrom® Steroids kit allows the detection and dosage of Aldosterone and structurally related 15 Steroids (Aldosterone, Androstenedione, Cortisol, 11-Deoxycortisol, Cortisone, Corticosterone, 11-Deoxycorticosterone, Dehydroepiandrosterone (DHEA), Dehydroepiandrosterone sulfate (DHEAS), Estradiol, Progesterone, 17-OH-Progesterone, Testosterone, Dihydrotestosterone (DHT)). The presence of dexamethasone and methylprednisolone in blood samples does not influence the quantification of the abovementioned steroids. Nevertheless, since dexamethasone interferes with the detection of 21-Deoxycortisol, this steroid will not be quantified.

Upon specific participant's consent, any residual biological sample will be stored for a maximum of 50 years within the Fondazione IRCCS Policlinico POLI-MI Biobank for future no-profit studies concerning COVID-19.

Statistical methods

A full data management plan and a full statistical analysis plan (SAP) will be developed by the study statistician, revised and approved by the scientific board, prior to study start; the SAP will include details of

data review at the end of each stage, in particular data on rate of adverse events and decision rules for arm(s) or study termination in case of excess adverse event rate.

After SAP signature by the scientific board (i.e. prior to any statistical analysis), the study database will be frozen at the end of the first stage, i.e. enrolment of 45 patients in Group A, with complete follow-up.

In particular, if at the end of the first stage ≤32/45 patient in group A are discharged alive, the study will be terminated. If this constraint is not met, the study will proceed to the second stage for both arms, and the database unfrozen.

Any changes to the protocol-specified or SAP-specified planned analyses that are made after the database lock will be described in the clinical study report.

Descriptive statistics will be obtained for all variables assessed in the study population. Mean and standard deviation will be used for normally distributed variables, median and interquartile range for skewed distributions, proportions for categorical variables. Whenever relevant, 95% confidence intervals (95%CI) will be calculated.

The Intention to Treat (ITT) population will be all patients randomized. The modified Intention to Treat (mITT) population will be all patients randomised and administered at least one study treatment (as such, it will also represent the "safety" population). The per-protocol population will be all patients who complete at least 48 hrs of treatment.

Any comparison will be only exploratory and not confirmatory in nature. Therefore, we do not indicate a cut-off for statistical significance.

Groups will be compared by means of parametric or nonparametric tests for quantitative variables and Pearson's χ^2 test (Fisher exact test where appropriate) for categorical variables. In all cases, two-tailed tests will be applied.

The effect of canrenone on in-hospital mortality will also be described as number, proportion and 95% confidence intervals, in the following subgroups:

- age >65 years vs ≤65 years

- males and females

- history of arterial hypertension under treatment at T1

- mean blood pression >100 mmHg at T1

- basal [K^+]hematic cut-off ≥4 mM at T1

Variables associated to in-hospital mortality, beside study group, will be assessed by means of regression models, adjusting for study group. Results will be expressed as Relative Risks (for T1, T2, and T3), and Hazard Ratios (for T4), with the corresponding 95% confidence intervals and p-value. Also, the effect of canrenone in specific subgroups will be assessed by adding an interaction term between study group and the relevant

subgroup indicator variable, in logistic regression models. It is anticipated that these analyses would yield a low statistical power.

Data Monitoring and Quality

The trial will be internally monitored by a competent team, appointed by the Sponsor: the internal team will be in charge of periodically monitoring study data collection, subjects' medical records and eCRFs in accordance with current regulations, ICH-GCP standards, and respective local national government, and international regulations and guidelines. The duration, nature and frequency of such visits/contacts shall depend on the rate of recruitment, the quality of the documents in the possession of the centre, and its adherence to the protocol.

Through these contacts, the monitor must:
- control and evaluate the progress of the study
- examine the collected data
- conduct Source Document Verification (SDV)
- identify and grade (critical, major, minor) possible deviations from the approved protocol and implement Corrective and Preventive Action plan (CAPA) whenever required.

The aims of the monitoring activity are to verify that:
- the rights and well-being of the subject are respected
- the study data are accurate, complete and verifiable by original documents
- the study is conducted in accordance with the protocol and any approved amendments, GCP and the applicable regulations.

The investigator must consent to:
- give the monitor direct access to all relevant documents;
- dedicate part of his time and the time of his staff to the monitor to discuss the results of the monitoring and any other possible aspects.

The monitor must also contact the centre before the beginning of the study to discuss the protocol and data collection procedures with the staff. The investigators will be responsible for all relevant data being entered into the electronic CRFs. The CRFs will be constructed in order to assure data quality with predefined values and ranges on all data entries. All participant data relating to the study will be recorded on eCRF.
The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF. The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents. Study documentation will be promptly and fully disclosed to the Promoter by the investigator upon request, and also shall be made available at the study site upon request for inspection, copying, review, and audit at reasonable times by representatives of the Promoter or any regulatory authorities. The investigator agrees to promptly take any reasonable steps that are requested by the Promoter or any regulatory authorities as a result of an audit or inspection to cure deficiencies in the study documentation and worksheets/CRFs.

Records and documents, including signed ICF, pertaining to the study must be retained by the investigator for 25 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Promoter. No records may be transferred to another location or party without written notification to the Promoter.

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. The investigator/institution should maintain adequate and accurate source documents and study records that include all pertinent observations on each of the site's participants. Source documents and data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (e.g., via an audit trail).

Harms

The Principal Investigator and Sponsor are responsible for reporting events that meet the AE or SAE definitions as described in this protocol. For each AE, Seriousness, Expectedness and Causality must be assessed by the Investigator/Sponsor in order to classify it properly.

Standard-of-care medications are to be considered as ReTNIMPs: as such, related Adverse Drug Reaction (ADR) Reporting will be managed as for Post-marketing signals.

SAE Classification - Definitions

Adverse event (AE): *"Any untoward or unfavourable medical occurrence in a clinical research study participant, including any abnormal sign (e.g. abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participants' involvement in the research, whether or not considered related to participation in the research".* An AE can therefore be any unfavourable 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.

Adverse events include:
- the exacerbation of a pre-existing pathology;
- an increase in the frequency or intensity of an episodic event or pre-existing condition;
- a condition occurring or diagnosed after the administration of the study drug, even if current before the start of the study;
- persistent diseases/symptoms at the baseline visit that worsen after the start of the study

Adverse events do not include:
- Medical or surgical procedures (e.g. surgery, endoscopy, tooth extraction, transfusions), but the condition requiring the procedure is an adverse event.
- Diseases or conditions present at the beginning of the study that have not worsened, but remained stable during the course of the study.
- Situations in which no unexpected adverse event has occurred (e.g. hospital admission for

elective cosmetic surgery/social problems).
- An overdose of drugs without onset of symptoms or associated signs.
- Laboratory abnormalities deemed by the investigator to be of no clinical significance.

Adverse Drug Reaction (ADR):

a) For the IMP: *all noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions.* The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.
b) For ReTNIMPs: *a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for modification of physiological function.*

Serious Adverse Event (SAE): *A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose:*

- *results in death,*
- *is life-threatening (patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.*
- *requires inpatient hospitalisation or prolongation of existing hospitalisation,*
- *results in persistent or significant disability/incapacity,*
- *is a congenital anomaly/birth defect*

The definition "serious" is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Suspected Unexpected Serious Adverse Event (SUSAR): *an adverse event, which is assessed by the sponsor and the study investigator as being unexpected, serious and as having a reasonable possibility of a causal relationship with the IMP.* Sponsor and/or Investigator are responsible to ensure that only serious ADRs with "reasonable causal relationship" (SAR) are assessed for expectedness and considered for SUSAR reporting.

SAE Classification – Assessments

Assessment of Causality: for each AE, the investigator must define the causal relationship between the event and the IMP administration. Causality will be recorded as:

- *Likely related*: event or laboratory test abnormality, with plausible and reasonable time relationship to drug intake; Unlikely to be attributed to disease or other drugs;
- *Possibly related:* Event or laboratory test abnormality, with reasonable time relationship to drug intake; Could also be explained by disease or other drugs; Information on drug withdrawal may be lacking or unclear;

- *Unlikely related:* Event or laboratory test abnormality with a time to drug intake that makes a relationship improbable; Disease or other drugs provide plausible explanations.

AE will be considered "IMP-related" in case the causal relationship drug-AE is classified as both likely or possibly related.

Assessment of Expectedness: an ADR will be considered expected if listed in its SmPC. If the nature, intensity or specificity of the ADR is not consistent with the applicable product information, it will be classified as unexpected.

Assessment of Intensity: Intensity of the AE will be assessed according to the NCI CTCAE, version 5. Any AE that changes CTCAE grade over the course of a given episode will have each change of grade recorded on the AE CRFs/worksheets.

• *Grade 1:* Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
• *Grade 2*: Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).
• *Grade 3:* Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
• *Grade 4:* Life threatening consequences; urgent intervention indicated.
• *Grade 5:* Death related to AE.

Safety Reporting

All ***AEs*** occurring after the randomization until 3 days ($t_{1/2}$ canrenone = 18 hrs; upon excretion, presumably within 72 hrs, no long-term adverse events related to canrenone are conceivable) after the end of IMP administration will be reported as AE in patient's record and in the study eCRF and graded according to the corresponding CTCAE term (Version 5). Any medical condition detected at screening visit will be considered basal condition and not reported as AE. Any change in severity of an AE will be managed as an update of the previous safety signal and not a new one.

All ***SAEs*** should be reported immediately to the Sponsor except for those that are expected according to the Summary of product Characteristics (SmPC) and study type. ADRs with severity and/or specificity and/or nature (defined as for CTCAE, version 5) are not consistent with SmPC will be also defined as unexpected. The Investigator notify every SAE within no more 24h after becoming aware of the event. The immediate reports should be followed promptly by detailed, written reports using the SAE Form. The immediate and follow-up reports should identify subjects by unique code numbers assigned to the trial subjects rather than by the subjects' names, personal identification numbers, and/or addresses. The Investigator as well as the Sponsor are required to assess causality and expectedness. All SAEs must be reported in patient's clinical record and in study eCRF. If the SAE is identified by investigators as a SUSAR, investigator must be reported immediately (within 24 hours) notification to the sponsor through CIOMS form.

Death is a likely event among severe ARDS patients: approximately 18% of patients are expected to die during hospitalization; thus, deaths will not be reported as SAE except in the case a causal relationship with the administration of the IMP is conceivable. Cases of death will be recorded in the eCRF since they represent the primary endpoint of the study. The investigator should supply the sponsor and the IEC with any additional requested information (e.g., autopsy reports and terminal medical reports).

Since the combination of MRA with Low Molecular Weight Heparin may arise the risk of hyperkalemia, the electrolytic balance will be thoroughly monitored and classified as Adverse Events of Special Interest (***AESI***); thus, hyperkalemia defined as $[K^+]^{hematic}$ > 5.1 mEq/L, whenever detected, will be promptly reported to Sponsor and no more than 24h after the Investigator becoming aware of the event. IMP administration will be promptly discontinued in case of severe hyperkalemia ($[K^+]^{hematic}$> 5.1 mEq/L) and/or hyponatriemia ($[Na^+]^{hematic}$< 126 mEq/L). Severity of hyperkalemia will be classified based on CTCAE version 5.0:

- Grade 1: >ULN - 5.5 mmol/L
- Grade 2: >5.5 - 6.0 mmol/L;
- Grade 3: >6.0 - 7.0 mmol/L;
- Grade 4: >7.0 mmol/L;
- Grade 5: Death

During the course of the study, all AEs and SAEs should be proactively followed up for each subject. Every effort should be made to obtain a resolution for all events, even if the events continue after discontinuation/study completion. The investigator is responsible for following all SAEs until resolution, until the subject returns to baseline status, or until the condition has stabilized with the expectation that it will remain chronic, even if this extends beyond study participation.

The *Sponsor* is responsible for the ongoing safety evaluation of the IMP. The Sponsor will:

- review all adverse events and issue queries directly to the Investigator reporting the event;
- determine if an event has to be considered as a SUSAR by providing supplementary considerations on seriousness, causality and expectedness;
- promptly notify all concerned investigators, the regulatory authority and IEC of findings that could affect the safety of subjects, impact the conduct of the trial, or alter the IEC's approval/favourable opinion to continue the trial;
- report all fatal or life-threatening SUSARs to Eudravigilance through the EVCTM, to all participating Investigators, the competent authority and to the Ethics Committee, as soon as possible and in any case no later than 7 days after knowledge by the sponsor of such a case, and that relevant follow-up information is subsequently communicated within an additional 8 days (According to the 2011/C 172 / 01 guidance); other SUSARs shall be reported within 15 days;
- provide an annual Development Safety Update Report (DSUR) within 60 days upon Data Lock Point, including all Serious Adverse Events occurring in the study, to the Regulatory Agency, all participating Investigators, and to the Ethical Committees of participating centres.

NOTIFICATIONS of AE, SAE, SUSAR

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Safety Analyses

AEs will be coded using the current version of Medical Dictionary for Regulatory Activities (MedDRA) and will be classified by system organs classes. Safety will be summarized by treatment group. Relationship to treatment, as well as severity will also be summarized.

Auditing

An internal team will be in charge of annual audits to inspect study data, subjects' medical records and eCRFs in accordance with current regulations, ICH-GCP standards, and respective local national government, and international regulations and guidelines. In its capacity as Promoter, the Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico can carry out a quality control audit at its discretion. In this case, the investigator must consent to give the auditor direct access to all relevant documentation and dedicating part of his time and the time of his staff to the auditor to discuss the results of the monitoring and any other possible aspects. Furthermore, the Regulatory Authorities (Italian Medicines Agency – AIFA) may also inspect the study. In this case, the investigator must consent to give the inspector direct access to all the relevant documentation and dedicating part of his time and the time of his staff to the inspector to discuss the results of the monitoring and any other possible aspects.

Ethics and dissemination

Research ethics approval

This study will be conducted in accordance with the rules of the ICH/GCP (International Conference of Harmonization/Good Clinical Practice) and all applicable laws; in accordance with the ethical principles that have their origin in the Declaration of Helsinki and with the respect to the European clinical practice, in compliance with all international guidelines and national law regulation in Italy.
The protocol and the informed consent document must be submitted to the Independent Ethics Committee (IEC) for review and will receive IEC approval/favourable opinion before initiation of the study. A progress report is sent to the IEC at least annually, and a summary of the study's outcome is sent at the end of the study.

Ethical and scientific quality standard are needed for designing, conducting, recording and reporting trials that involve the participation of human subjects. Training activities aimed at creating a solid team that share clinical and research expertise will be carried out. The present study does not imply any potential specific risks for the subjects (see Risk/Benefit considerations for details). Based on previous literature, canrenone is considered a safe drug with a low rate of mild side effects.

Protocol amendments

During the study, any amendments to the protocol must also be approved by IEC.

Amendament No.	***Date***	***Description of main changes***

Informed Consent Form

The investigator or medically qualified designee (defined in the Study Delegation Log) must obtain Informed Consent Form (ICF) from each potential participant or each participant's legally acceptable representative prior to participating in a clinical study. According to the recommendations of the Declaration of Helsinki and local regulations, each patient will be adequately informed about the aims, methods, expected benefits, potential risks and problems related to the study, using lay and comprehensive terms. Moreover, patients will be informed of their right to refuse consent to the use of their sensitive data or to withdraw it at any time, without having any effect on their medical care. The study staff will be available for any further questions concerning the trial.

Participants enrolled will be required to consent to the deidentified use of personal data for the study. Information form and the module for the acquisition of informed consent for the handling of sensitive data will be given to the patient.

The investigator will fulfil the current regulations for research and documentation of informed consent, the standards of Good Clinical Practice and the ethical principles derived from the Declaration of Helsinki. The approval by the Ethics Committee will be required whether an update of the informed consent form will be needed during the study.

The patient will have all the time necessary for the evaluation of the information received before providing their informed consent to the use of sensitive data. The investigator will have to obtain spontaneous informed consent in writing by the patient, before using them in any way for the study. The written consent to the handling of sensitive data must be subscribed by the date and signature of the patient and by the investigator's or his/her representative's ones. The investigator has to give the patient a signed copy of informed consent; the original form will be retained with the other documents of the study protocol; the module for the acquisition of informed consent to the treatment of sensitive data will be attached to the clinic folder.

Consent must be documented by the participant's dated signature or by the participant's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion. A copy of the signed and dated consent form should be given to the participant before participation in the study.

The initial ICF, any subsequent revised written ICF, and any written information provided to the participant must receive the Institutional Review Board/Independent Ethics Committee's (IRB/IEC's) approval/favourable opinion in advance of use. The participant or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the participant's willingness to continue participation in the study. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the participant's dated signature or by the participant's legally acceptable representative's dated signature.

Unconscious patients will not be enrolled in this trial.

Insurance

Ad hoc study insurance requirement has been waived by AIFA for no-profit studies on COVID19 drugs (AIFA notice, "Clinical trials' management in Italy during the COVID-19 emergency", Version 3, 17 September 2020).

Confidentiality

The personnel bound by professional secret must maintain the confidentiality of all personal identity or personal medical information (according to the confidentiality and personal data protection rules). The confidentiality of records that could identify subjects should be protected, only initials of the name and the first name will be registered with an inclusion coded number for the study (no name nor address nor identifying data). At enrolment, a univocal study ID code will be assigned to each participant. The file that mates ID code and personal identifiers will be stored separately on a password-protected computer. Study database will be also protected by password with access limited to the PI or qualified designee (defined in the Study Delegation Log). Data deidentification will assure that personnel consulting the study database will not be able to trace back participants' identity.

Declaration of interests

The Principal Investigator (PI) and study staff have no conflicts of interests to declare. A specific declaration is provided by the PI excluding any financial and/or competing conflicts of interest with the Marketing Authorization Holder of canrenone.

Access to data

Data in the eCRF will only be accessed by authorized study staff.

The full study database will be stored in the REDCap platform of the Fondazione and will be available for inspection and future analysis upon request to the principal investigator, and subject to approval by the Ethical Committee of the Fondazione.

Ancillary and post-trial care

After discharge, patients will be followed up according to routine care, at the out-patient clinic organized at the Fondazione. This includes 3- 6- and 12-month follow-up.

Dissemination and Publication policies

The present trial will be conducted respecting rules of European legislation for interventional clinical trials on medicinal products. Details of this trial will be registered on the European Union Drug Regulating Authorities Clinical Trials Database (EudraCT) and on clinicaltrial.gov portal. The results of this study will be posted in EudraCT EMA/clinicaltrial.gov portals with 12 months after the end of the trial. Results will also be published in peer-reviewed international journals or presented at scientific meetings. The Promoter will comply with the requirements for publication of study results. Communications, reports, and publication of the results of the study will be under the responsibility of the principal investigator of the study. A summary of the results of the study will written and provided on request of the participating patients.

Authorship policy will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements and will follow the ICMJE criteria, and authorship will be based on the following 4 criteria:

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
- Drafting the work or revising it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- 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

First author will be the Principal Investigator, and last author the guarantor of the study.

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