

**suPAR-GUIDED ANAKINRA TREATMENT FOR VALIDATION OF THE RISK AND EARLY MANAGEMENT OF SEVERE RESPIRATORY FAILURE BY COVID-19: THE SAVE OPEN-LABEL, NON-RANDOMIZED SINGLE-ARM TRIAL**

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**STUDY PROTOCOL**

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## DISCLOSURE OF PRINCIPAL INVESTIGATOR

**Protocol Study Title:** suPAR-GUIDED ANAKINRA TREATMENT FOR VALIDATION  
OF THE RISK AND EARLY MANAGEMENT OF SEVERE RESPIRATORY FAILURE  
BY COVID-19: THE SAVE OPEN-LABEL, NON-RANDOMIZED SINGLE-ARM TRIAL

The herein protocol became known to myself by the Study Sponsor. I understand  
that the protocol remains as yet unpublished; I certify that all disclosed information to  
myself for this protocol will remain strictly confidential.

The Principal Investigator,

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Print Name

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Signature

Date

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## LIST OF ABBREVIATIONS

AE: adverse event

CI: confidence interval

COVID-19: Coronavirus 2019 disease

CPAP: continuous positive airway pressure

EDTA: ethylene-diamene-tetracetic acid

FiO<sub>2</sub>: fraction of inspired oxygen

HR: hazard ratio

IL: interleukin

IFN: interferon

IV: intravenous

PBMCs: peripheral blood mononuclear cells

pO<sub>2</sub>: partial oxygen pressure

RCT: randomized clinical trial

SAE: serious adverse event

SRF: severe respiratory failure

SOFA: sequential organ failure assessment

suPAR: soluble urokinase plasminogen activator receptor

TNF $\alpha$ : tumor necrosis factor-alpha

WHO: World Health Organization

## SYNOPSIS

<b>Aim</b>	Recent data coming from the Hellenic Sepsis Study Group reveal that suPAR levels $\geq 6$ ng/ml are early found among patients who will eventually develop SRF by COVID-19 with positive predictive value more than 80%. This signifies that an early pro-inflammatory reaction has been started in the lung. It is postulated that early anakinra treatment in these patients may halt this reaction and prevent development of SRF.
<b>Design</b>	Prospective, multicenter, open-label, single-arm trial
<b>Inclusion criteria</b>	<ul style="list-style-type: none"> <li>• Age equal to or above 18 years</li> <li>• Male or female gender</li> <li>• In case of women, unwillingness to remain pregnant during the study period.</li> <li>• Written informed consent provided by the patient or by one first-degree relative/spouse in case of patients unable to consent</li> <li>• Confirmed infection by SARS-CoV-2 virus</li> <li>• Findings in chest-X-ray or in chest computed tomography compatible with lower respiratory tract infection</li> <li>• Plasma suPAR <math>\geq 6</math>ng/ml</li> </ul>
<b>Intervention</b>	Patients will receive once daily 100mg of anakinra subcutaneously for ten days. The drug should be administered on the same time $\pm 2$ hours every day. All other administered drugs are allowed. In case the patient is discharged home before the completion of 10 days of treatment, it is at the discretion of the investigator to suggest treatment continuation at home. In case such a decision is taken, the patient will be provided the required number of pre-filled syringes for daily self-injection. For patients with lymphopenia, it is at the discretion of the investigator to administer one single tablet of 80mg/400mg trimethoprim/sulfamethoxazole as prophylaxis.
<b>Primary study endpoint</b>	The primary study endpoint is the rate of patients who will develop SRF (see appendix V for definition) until day 14. Patients dying before study visit of day 14 are considered achieving the primary endpoint.
<b>Secondary study endpoints</b>	<ul style="list-style-type: none"> <li>• Comparison of the primary endpoint with comparators receiving standard-of-care treatment</li> <li>• Change of scoring for respiratory symptoms between days 1 and 7</li> </ul>

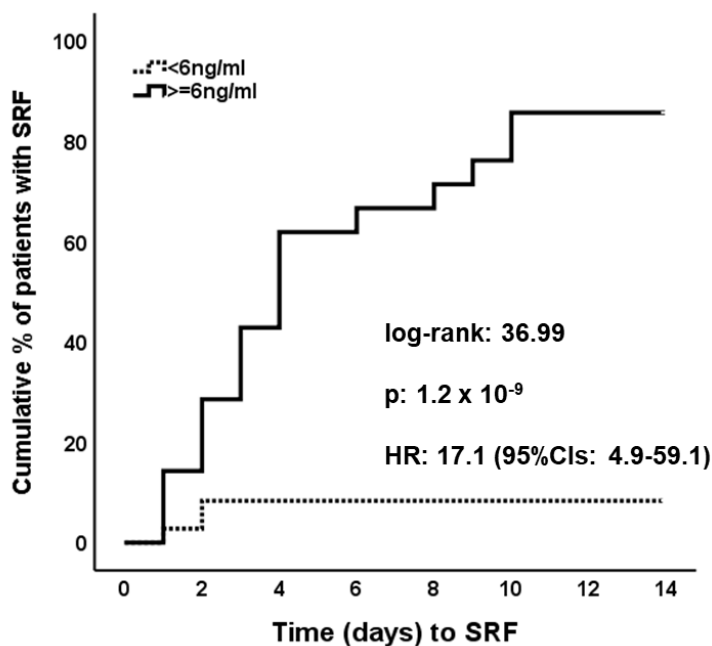
	<ul style="list-style-type: none"> <li>• Change of scoring for respiratory symptoms between days 1 and 14</li> <li>• Change of SOFA score between days 1 and 7</li> <li>• Change of SOFA score between days 1 and 14</li> <li>• Change of Peripheral Blood Mononuclear Cells' (PBMCs) functionality between days 1 and 7</li> <li>• Change of gene expression between days 1 and 7 (transcriptional, proteomic and metabolomic analysis)</li> <li>• Change of plasma inflammatory mediators between days 1 and 7</li> <li>• 30-day mortality</li> <li>• 90-day mortality</li> <li>• Safety of anakinra</li> <li>• Association between the time interval from hospital admission until start of anakinra and the incidence of SRF</li> <li>• Association between viral load and the incidence of SRF under anakinra treatment</li> <li>• Association between radiological opacities in chest computed tomography and the incidence of SRF under anakinra treatment</li> <li>• Association of the efficacy of anakinra for subgroups of patients; the studied subgroups will be the quartiles of the respiratory ratio (<math>pO_2/FiO_2</math>) at admission; the main comorbidities; the WHO classification</li> </ul>
<p><b>Number of patients</b></p>	<p>The study power calculation is based on the hypothesis that 60% of patients with lower respiratory tract infection due to COVID-19 and suPAR<math>\geq</math>6 ng/ml upon hospital admission, will develop SRF. To decrease this to 45% with power of 90% at a 5% level of significance, two hundred-sixty (260) patients need to be enrolled. Making the hypothesis that patients who start anakinra the first two days from hospital admission (early start) will develop SRF at 15% and those who start later will develop SRF at 25% (late start), 1000 patients are need to demonstrate this with 90% power at the 5% level of significance.</p>
<p><b>Duration</b></p>	<p>2 years</p>
<p><b>Interim Analysis</b></p>	<p>Interim analysis will be performed after completion of 30-day follow up of the first 130 patients. A comparison with a group of comparators receiving standard-of-care treatment will be performed.</p>

## BACKGROUND

The major hurdle of COVID-19 is the early recognition of the patients at high risk for the development of severe respiratory failure (SRF). If this can be achieved early, then appropriate immunomodulatory treatment may be administered to prevent development of SRF. This scenario is extremely visionary since it prevents the development of the major fatal consequence of Covid-19 but also alleviates the heavy medical and financial burden of Intensive Care Unit (ICU) admission.

Current evidence suggests that SARS-CoV-2 activates endothelial function which leads to over-production of D-dimers<sup>1,2</sup>. uPAR (urokinase plasminogen activator receptor) is anchored to the cell membranes of the lung endothelial cells. As result of the activation of kallikrein, uPAR is cleaved and enters the systemic circulation as the soluble counterpart suPAR<sup>3</sup>. Preliminary unpublished data from 57 Greek patients hospitalized after March 1<sup>st</sup> 2020 in Greek hospitals due to pneumonia by confirmed SARS-CoV-2 infection showed that those with suPAR admission levels  $\geq 6$  ng/ml had greater risk for the development of SRF within 14 days than patients with suPAR less than 6ng/ml (Figure 1). The sensitivity of suPAR to detect these patients was 85.9% and the positive predictive value 85.9%. It needs to be outscored that all 21 Greek patients with suPAR $\geq$  6ng/ml were under standard-of-care treatment, which at that moment consisted of hydroxychloroquine and azithromycin. These data were confirmed in 15 patients hospitalized for pneumonia by SARS-CoV-2 in Rush Medical Center at Chicago<sup>4</sup>. A recent publication of 881 patients from the ISIC database verified these results<sup>5</sup>.

This prognostic ability of suPAR for unfavourable outcome is not presented for the first time; in the TRIAGE III trial that was conducted among 4,420 admissions in the emergency department in Denmark the interquartile range of suPAR was between 2.6 and 4.7 ng/ml in 30-day survivors and between 6.7 and 11.8 ng/ml in 30-day non-survivors<sup>6</sup>. Previous data from the Hellenic Sepsis Study Group on 1,914 patients clearly shows a high prognostic utility of admission suPAR for 28-day mortality<sup>7</sup>.



**Figure 1 Time to progression into SRF**

Patients are divided into those with suPAR ≥6 ng/ml on the day of hospital admission (n= 21) and into those with suPAR less than 6ng/ml on the day of hospital admission (n=36). The log-rank test and the p-value of comparison are provided

Abbreviations CI: confidence interval; HR: hazard ratio

It is obvious that suPAR can early identify the start of such a type of inflammatory process in the lung parenchyma that will soon be intensified. A recent publication has shown that this is due to the early release of IL-1 $\alpha$  from lung epithelial cells that are infected by the virus. This IL-1 $\alpha$  acts as a promoting factor that stimulates the production of IL-1 $\beta$  and of a further cytokine storm from alveolar macrophages<sup>8</sup>.

Anakinra is the only marketed product that inhibits both IL-1 $\beta$  and IL-1 $\alpha$  and hence it is able to block an inflammatory response early on and to prevent the downstream inflammatory cascade. suPAR can be used as the biomarker tool to indicate patients with COVID-19 pneumonia in risk of SRF and in whom early start of anakinra may prevent development of SRF.

Anakinra is a safe drug that has been licensed for chronic subcutaneous administration in rheumatoid arthritis, refractory gout and chronic auto-inflammatory disorders<sup>9</sup>. The safety profile was further proven when it was administered in two



randomized clinical trials where more than 1,500 critically ill patients with severe sepsis were intravenously treated<sup>10,11</sup>.

## **AIM OF THE STUDY**

In the SAVE study patients with lower respiratory tract infection by SARS-CoV-2 at high risk of SRF will be traced using suPAR. They will then start early treatment with anakinra in the aim to prevent the development of SRF.

## **STUDY DESIGN**

This will be a prospective open-label non-randomized study that will take place for 24 months in study sites in Greece (Appendix I). The study protocol will be approved by the National Ethics Committee of Greece and by the National Organization for Medicines of Greece. The study will be registered at Clinicaltrials.gov before enrolment of the first patient.

## **Study population**

Patients who meet ALL the following inclusion criteria and who do not meet any of the following exclusion criteria are allowed to be enrolled:

### Inclusion criteria

1. Age equal to or above 18 years
2. Male or female gender
3. In case of women, unwillingness to remain pregnant during the study period.
4. Written informed consent provided by the patient or by one first-degree relative/spouse in case of patients unable to consent
5. Confirmed infection by SARS-CoV-2 virus
6. Findings in chest-X-ray or in chest computed tomography compatible with lower respiratory tract infection
7. Plasma suPAR  $\geq 6$ ng/ml

### Exclusion criteria

- Age below 18 years
- Denial for written informed consent

- Any stage IV malignancy
- Any do not resuscitate decision
- Any  $pO_2/FiO_2$  ratio less than 150
- Any need for CPAP or mechanical ventilation
- Any primary immunodeficiency
- Less than 1,500 neutrophils/mm<sup>3</sup>
- Known hypersensitivity to anakinra
- Known hypersensitivity to trimethoprim/sulfamethoxazole
- Known deficiency of the G-6PD enzyme
- Oral or IV intake of corticosteroids at a daily dose equal or greater than 0.4 mg prednisone for a period greater than the last 15 days.
- Any anti-cytokine biological treatment the last one month
- Severe hepatic failure
- Severe renal failure
- Pregnancy or lactation. Women of child-bearing potential will be screened by a urine pregnancy test before inclusion in the study

### **Screening for eligibility**

No study related procedure will be performed prior to obtaining written informed consent form. Screening follows these next steps:

- Step 1: The patient is screened for the exclusion criteria. If he meets any of them, he cannot be enrolled. If he does not meet any of them, he remains eligible and screening proceeds to Step 2
- Step 2: The patient is screened for inclusion criteria 1 to 6. If he meets these criteria, he remains eligible and screening proceeds to Step 3.
- Step 3: 18 ml of whole blood is drawn after venipuncture of one forearm vein under aseptic conditions and collected into one EDTA-coated and one non-EDTA-coated tube. The first tube is centrifuged for plasma collection. A commercialized quick blood test with suPARnostic® Quick Triage (Virogates S/A, Blokken 45, 3460 Birkerød, Denmark) will take place, to determinate in a very short time (20 min) suPAR levels in human EDTA-plasma. The sample (100 µl of plasma) will be

incubated and handled, according to the manufacturer's instructions, attached to a provided reader and the results will be displayed through LF Software. The measurement is the result of a lateral flow immunoassay (LFIA) and constitutes a quantitative measurement (in ng/ml) of plasma suPAR levels, provided that suPAR values are detected within the range of 2-15 ng/ml, to be considered accurate. If suPAR is found  $\geq 6$  ng/ml, the patient can be enrolled in the study. Blood analysis from blood collected in the second tube will be done as described at the section Laboratory Procedures

### ***Intervention***

Patients will receive 100mg of anakinra subcutaneously once daily for ten days. The drug should be administered on the same time  $\pm 2$  hours every day. In case the patient is discharged home before the completion of 10 days of treatment, it is at the discretion of the investigator to suggest treatment continuation at home. In case such a decision is taken, the patient will be provided the required number of pre-filled syringes for daily self-injection. In this case, the patient should return the empty used syringes within 30 days. For patients with lymphopenia, it is at the discretion of the investigator to administer one single tablet of 80mg/400mg trimethoprim/sulfamethoxazole as prophylaxis. In that case trimethoprim/sulfamethoxazole is given on the same day and time as anakinra.

### **Study drug**

The active study drug i.e. anakinra will be provided in the form of pre-filled ready-to-use syringes. All syringes need to be stored at 2-8°C at the study site at a refrigerator with recording of temperature. In case recording indicates deviation of temperature below 0°C or above 10°C for more than a day, stored syringes need to be replaced by the Sponsor. At the exterior of each syringe there will be a letter and a 4-digit number. The letter refers to the study site, the first two digits of the number refer to the serial number of enrolled patient at the respective study site and the last two digits refer to the day of treatment. For example, the code A0102 refers to study site A, patient number 01 at that study site and treatment day 2. In case of patients discharged earlier and continuing the drug by self-injection, pre-filled syringes will be provided in a cooling bag.

The adverse events of anakinra are classified as very common ( $\geq 1/10$ ), common ( $\geq 1/100$  to  $< 1/10$ ) and uncommon ( $\geq 1/1,000$  to  $< 1/100$ ) and they are presented in the Table below<sup>9</sup>:

MedDRA Organ System	Frequency	Undesirable Effect
Infections and infestations	Common	Serious infections
Blood and lymphatic system disorders	Common	Neutropenia Thrombocytopenia
Immune system disorders	Uncommon	Allergic reactions including anaphylactic reactions, angioedema, urticaria and pruritus
Nervous system disorders	Very common	Headache
Hepatobiliary disorders	Uncommon	Hepatic enzyme increased
	Not known	Non-infectious hepatitis
Skin and subcutaneous tissue disorders	Very common	Injection site reaction
	Uncommon	Rash
Laboratory	Very common	Blood cholesterol increased

The adverse events of trimethoprim/sulfamethoxazole are classified as very common ( $\geq 1/10$ ), common ( $\geq 1/100$  to  $< 1/10$ ) and uncommon ( $\geq 1/1,000$  to  $< 1/100$ ) and they are presented in the Table below<sup>12</sup>:

MedDRA Organ System	Frequency	Undesirable Effect
Infections and infestations	Common	Overgrowth fungal
	Very rare	Pseudomembranous colitis
Blood and lymphatic system disorders	Very rare	Leukopenia, neutropenia, thrombocytopenia, megaloblastic anaemia, aplastic anaemia, haemolytic anaemia, methaemoglobinaemia, eosinophilia, haemolysis in certain susceptible G-6-PD deficient patients.
Immune system disorders	Very rare	Serum sickness, anaphylactic reaction, allergic myocarditis, hypersensitivity vasculitis resembling Henoch-Schoenlein purpura, periarteritis nodosa, systemic lupus erythematosus.
Metabolism and nutrition disorders	Very common	Hyperkalaemia
	Very rare	Hypoglycaemia, hyponatraemia, decreased appetite, metabolic acidosis
Psychiatric disorders	Very rare	Depression, hallucination.
	Not known	Psychotic disorder
Nervous system disorders	Common	Headache.
	Very rare	Aseptic meningitis, convulsions, neuropathy peripheral, ataxia, dizziness.

Ear and labrynth disorders	Very rare	Vertigo, tinnitus
Eye disorders	Very rare	Uveitis
Respiratory, thoracic and mediastinal disorders	Very rare	Cough, dyspnoea, lung infiltration
Gastrointestinal disorders	Common	Nausea, diarrhoea
	Uncommon	Vomiting
	Very rare	Glossitis, stomatitis, pancreatitis
Hepatobiliary disorders	Very rare	Increase of aminotransferases, bilirubin increase, cholestatic jaundice, hepatic necrosis
Skin and subcutaneous tissue disorders	Common	Rash
	Very rare	Photosensitivity reaction, angiodema, dermatitis exfoliative, fixed drug eruption, erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis
	Not known	Drug reaction with eosinophilia and systemic symptoms (DRESS)
Musculoskeletal and connective tissue disorders	Very rare	Arthralgia, myalgia.
Renal and urinary disorders	Very rare	Renal impairment

## Patients' visits and interventions (Appendix II)

### Day 1

This visit will take place on the morning of the day of the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-morbidities, co-administered drugs, past-history, SOFA score (Appendix III), absolute blood cell count and differential (if available) and biochemistry (if available)
- Recording of the % degree of lung involvement using the opacities of chest computed tomography
- Collection of sample from the upper airways for RT-PCR testing for SARS-CoV-2 to detect the viral load
- Recording of the time interval from hospital admission until start of anakinra
- Scoring of the respiratory symptoms (Appendix IV).
- Administration of the study drug
- Recording of adverse events (AE) and severe adverse events (SAE)

### Day 2

This visit will take place on the morning of the second day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available) and biochemistry (if available)
- Recording of the presence or not of SRF (Appendix V)
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug
- The visit may take place by phone call or through the internet in case of hospital discharge

### Day 3

This visit will take place on the morning of the third day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available) and biochemistry (if available)
- Recording of the presence or not of SRF (Appendix V)
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug
- The visit may take place by phone call or through the internet in case of hospital discharge

### Day 4

This visit will take place on the morning of the fourth day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available) and biochemistry (if available)
- Recording of the presence or not of SRF (Appendix V)
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug

- The visit may take place by phone call or through the internet in case of hospital discharge

#### Day 5

This visit will take place on the morning of the fifth day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available) and biochemistry (if available)
- Recording of the presence or not of SRF (Appendix V)
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug
- The visit may take place by phone call or through the internet in case of hospital discharge

#### Day 6

This visit will take place on the morning of the sixth day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available) and biochemistry (if available)
- Recording of the presence or not of SRF (Appendix V)
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug
- The visit may take place by phone call or through the internet in case of hospital discharge

#### Day 7

This visit will take place on the morning of the seventh day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, SOFA score (Appendix III), absolute blood cell count and differential (if available) and biochemistry (if available)
- Scoring of the respiratory symptoms (Appendix IV).
- Recording of the presence or not of SRF (Appendix V)
- Recording of adverse events (AE) and severe adverse events (SAE)

- Sampling of 15 ml of venous blood. Blood analysis will be done as described at the section Laboratory Procedures.
- Administration of the study drug
- The visit may take place by phone call or through the internet in case of hospital discharge. In that case, no blood sampling will take place.

### Day 8

This visit will take place on the morning of the eighth day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available) and biochemistry (if available)
- Collection of sample from the upper airways for RT-PCR testing for SARS-CoV-2 to detect the viral load
- Recording of the presence or not of SRF (Appendix V)
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug
- The visit may take place by phone call or through the internet in case of hospital discharge

### Day 9

This visit will take place on the morning of the ninth day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available) and biochemistry (if available)
- Recording of the presence or not of SRF (Appendix V)
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug
- The visit may take place by phone call or through the internet in case of hospital discharge

### Day 10



This visit will take place on the morning of the tenth day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available) and biochemistry (if available)
- Recording of the presence or not of SRF (Appendix V)
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug
- The visit may take place by phone call or through the internet in case of hospital discharge

#### Day 14

This visit will take place on the morning of the 14<sup>th</sup> day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, SOFA score (Appendix III), absolute blood cell count and differential (if available) and biochemistry (if available)
- Scoring of the respiratory symptoms (Appendix IV).
- Recording of the presence or not of SRF (Appendix V)
- Recording of adverse events (AE) and severe adverse events (SAE)
- The visit may take place by phone call or through the internet in case of hospital discharge

### **LABORATORY PROCEDURES**

Blood samples will be collected in EDTA-coated and not-EDTA-coated tubes and in PAXgene tubes. Samples will be transferred on the day of collection to the central Lab, which is the the Laboratory of Immunology of Infections at the 4<sup>th</sup> Department of Internal Medicine at ATTIKON University General Hospital. Analysis will be performed in the central Lab and in another European university Lab outside Greece.

Mediators of inflammation and suPAR will be measured in plasma and serum. Among measured mediators will be sIL-2R, IL-8 triglycerides as well as coagulation and fibrinolysis mediators.

All stored PAXgene tubes will be subjected to transcriptomic analysis. Part of the collected plasma will be stored at -80°C for proteomic and metabolomic analysis.

Peripheral Blood Mononuclear Cells (PBMCs) will be isolated after gradient centrifugation of whole blood over Ficoll. After serial washing, counting and exclusion of dead cells, they will be stimulated with purified ligands of SARS-CoV-2 for the production of TNF $\alpha$ , IL-1 $\beta$ , IL-6, IL-10, IL-17, IL-22 and IFN $\gamma$ .

## **STUDY ENDPOINTS**

### **Primary study endpoint**

The primary study endpoint is the rate of patients who will develop SRF (see Appendix V for definition) until day 14. Patients developing SRF or dying before study visit of day 14 are considered achieving the primary endpoint.

### **Secondary study endpoints**

- Comparison of the primary endpoint with comparators receiving standard-of-care treatment
- Change of scoring for respiratory symptoms between days 1 and 7
- Change of scoring for respiratory symptoms between days 1 and 14
- Change of SOFA score between days 1 and 7
- Change of SOFA score between days 1 and 14
- Change of Peripheral Blood Mononuclear Cells' (PBMCs) functionality between days 1 and 7
- Change of gene expression between days 1 and 7 (transcriptional, proteomic and metabolomic analysis)
- Change of plasma inflammatory mediators between days 1 and 7
- 30-day mortality
- 90-day mortality
- Safety of anakinra
- Association between the time interval from hospital admission until start of anakinra and the incidence of SRF
- Association between viral load and the incidence of SRF under anakinra treatment
- Association between radiological opacities in chest computed tomography and the incidence of SRF under anakinra treatment

- Association of the efficacy of anakinra for subgroups of patients; the studied subgroups will be the quartiles of the respiratory ratio ( $pO_2/FiO_2$ ) at admission; the main comorbidities; the WHO classification

## NUMBER OF PATIENTS

The study power calculation is based on the hypothesis that 60% of patients with lower respiratory tract infection due to COVID-19 and  $suPAR \geq 6$  ng/ml upon hospital admission, will develop SRF. To decrease this to 45% with power of 90% at a 5% level of significance, two hundred-sixty (260) patients need to be enrolled. Making the hypothesis that patients who start anakinra the first two days from hospital admission (early start) will develop SRF at 15% and those who start later will develop SRF at 25% (late start), 1000 patients are need to demonstrate this with 90% power at the 5% level of significance.

## INTERIM AND STATISTICAL ANALYSIS

An interim analysis will be conducted after completion of the 30-day follow up of the first 130 patients, i.e. 50% of the number of patients needed to be enrolled as calculated by power analysis. As comparators will be used patients hospitalized with pneumonia by the SARS-CoV-2 virus in other departments of Greek hospitals in which full data collection is performed by the Hellenic Sepsis Study Group, after written informed consent. Data collection in these departments has been approved by the Ethics Committees of the respective hospitals. For the purpose of this analysis, patients receiving standard-of-care treatment (SOC) will be selected among the total patients whose data are fully collected in order to fulfill the same inclusion and exclusion criteria as patients enrolled in the SAVE trial. Propensity matching will be performed among comparators and 130 patients enrolled in the SAVE trial, so as the 130 comparators will be fully comparable to all below criteria:

- Age
- Comorbidities, as expressed by the Charlson's Comorbidity Index
- Admission severity scores namely pneumonia severity index (PSI), acute physiology and chronic health evaluation (APACHE) II score, SOFA score and WHO severity classification of COVID-19
- Co-administered treatment, such as azithromycin and dexamethasone

The primary endpoint will be expressed as percentage and 95% confidence intervals. Comparisons with historical cases will be done by the Fisher exact test. Any p-value below 0.05 will be considered significant.

## ADVERSE EVENTS

Adverse events (AEs) and Serious Adverse Events (SAEs) will be collected from baseline until the last patient's evaluation. Investigators should monitor subjects for adverse events and are responsible for recording ALL adverse events and serious adverse events occurring to a patient during the trial.

An adverse event is any undesirable medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. The time relationship is defined from the moment the AE occurs during therapeutic treatment until 30 days or 5 half-lives after treatment discontinuation. The adverse event may be a sign, a symptom, or an abnormal laboratory finding.

**Serious adverse events** (SAEs) must be reported to within 24 hours. If an adverse event meets any of the following criteria, it is considered SAE:

- **Life-threatening situation** The subject was at risk of death at the time of the adverse event/experience. It does not refer to the hypothetical risk of death if the AE were more severe or were to progress.
- **Inpatient hospitalization** or prolongation of existing hospitalization.
- **Persistent or significant disability/incapacity** Any AE having an outcome that is associated with a substantial disruption of the ability to carry out normal life functions, including the ability to work. This is not intended to include transient interruption of daily activities.
- **Congenital anomaly/birth defects** Any structural abnormality in subject's offspring that occurs after intrauterine exposure to treatment.
- **Important medical events/experiences** that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event/experience when, based upon appropriate medical judgment, **they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above**, i.e., death, a life-threatening

adverse event/experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Examples of such medical events/experiences include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

- **Spontaneous and elective abortions** experienced by study subject.

**A non-serious adverse event** is any untoward medical occurrence in a patient or subject who is administered a pharmaceutical product, and which does not necessarily have a causal relationship with this treatment. A non-serious adverse event is one that does not meet the definition of a serious adverse event given.

### **Grading of severity**

The severity of the adverse events shall be graded as:

- **Mild** the adverse event is transient and well tolerated by the patient
- **Moderate** the adverse events causes discomfort and affects the usual activities of the patient.
- **Severe** the adverse events affects the usual activities of the patient to an important degree and may cause disability or be life-threatening.

### **Relationship to the drug**

The investigator will use the following definitions to assess the relationship of the adverse event to study drug:

- **Probably Related:** The adverse event has a strong time relationship to the drug or relapses if re-induced, and another etiology is improbable or clearly less probable.
- **Possibly Related:** The adverse event has a strong time relationship to the drug and an alternative aetiology is as probable or less probable.
- **Probably not Related:** The adverse event has a slight or no time relationship to the drug and/or there is a more probable alternative aetiology.
- **Unrelated:** The adverse event is due to an underlying or concomitant disease or to another pharmaceutical product and is not related to the drug (no time relationship and a much more probable alternative aetiology).

If an investigator's opinion of possibly related, probably not related or not related to study drug is given, an alternate etiology must be provided by the investigator. Please note that a severe adverse event/experience is not necessarily serious, as the term severe is a measure of intensity while a serious adverse event is determined based on the aforementioned regulatory criteria. Individual un-blinding thought to be necessary for the management of an adverse event will be documented in the subject Case Report Form.

## **QUALITY CONTROL AND ASSURANCE**

Quality control and assurance checks are performed by sponsor in order to allow periodic review of adequacy of the study activities and practices and allow for revising such practices as needed so the data and process are maintained, the study meets the protocol and procedural requirements, and is reproducible.

Before enrolling any subject in this study, sponsor personnel and the investigator have to review the protocol, the IB, the CRFs and instructions for their completion, the procedure for obtaining informed consent and the procedure for reporting AEs and SAEs.

A qualified representative of the sponsor will monitor the conduct of the study by visiting the site and by contacting the site by telephone and e-mail. During these site visits, all source documents are reviewed, and information recorded in the CRFs is verified against them.

Besides routine monitoring, quality assurance will be documented through independent auditing of the quality control activities and where applicable, by regulatory authorities through inspections.

## **ETHICAL CONSIDERATIONS**

Prior to the initiation of this study, the study design will receive ethical, scientific, and where applicable, regulatory review. Investigators will conduct this study in accordance with the principles of the Declaration of Helsinki, GCP, and applicable regulatory requirements.

Regarding Informed Consent Form obtaining procedures, before any procedure specified in the protocol is performed, a subject must:

- Be informed of all pertinent aspects of the study and all elements of informed consent
- Be given time to ask questions and to consider the decision to participate
- Voluntarily agree to participate in the study
- Sign and date the updated and approved by IEC/REB ICF version.

## **PROTOCOL ADHERENCE AND AMENDMENTS**

Investigators ascertain that they will apply due diligence to avoid protocol deviations. All significant protocol deviations will be recorded and reported in the clinical study report (CSR). Any change or addition to the protocol can only be made in a written protocol amendment that must be approved and signed by the sponsor, health authorities where required, and the IEC/REB.

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## **APPENDIX I List of study sites**

- 1<sup>st</sup> Department of Internal Medicine, AHEPA University General Hospital of Thessaloniki (PI: Simeon Metallidis, Associate Professor of Internal Medicine and Infectious Diseases)
- 2<sup>nd</sup> University Department of Internal Medicine, IPPOKRATEION General Hospital of Athens (PI: Helen Sambatakou, Assistant Professor of Internal Medicine and Infectious Diseases)
- 3<sup>rd</sup> University Department of Internal Medicine, General Hospital of Chest Diseases of Athens I SOTIRIA, (PI: Garyfallia Poulakou, Assistant Professor of Internal Medicine and Infectious Diseases)
- Department of Internal Medicine, General Hospital of Chest Diseases of Athens I SOTIRIA (PI: Aikaterini Argyraki, Senior Registrar)
- COVID-19 Department, General Hospital of Attica SISMANOGLEIO-AMALIA FLEMING, (PI: Malvina Lada, Director of NHS)
- Department of Internal Medicine, I PAMMAKARISTOS Hospital, (PI: Ioannis Baraboutis, Director of NHS)
- Department of Internal Medicine, University General Hospital of Patras PANAGIA I VOITHIA, (PI: Charalambos Gogos, Professor of Internal Medicine)
- Department of Internal Medicine, University General Hospital of Larissa, (PI: George Dalekos, Professor of Internal Medicine)
- 2<sup>nd</sup> Department of Internal Medicine, University General Hospital of Alexandroupolis, (PI: Periklis Panagopoulos, Assistant Professor of Internal Medicine)
- Department of Clinical Therapeutics, ALEXANDRA General Hospital of Athens, (PI: Evangelos Kostis, Director of NHS)
- Department of Pulmonary Medicine, General Hospital of Kerkira, (PI: Ilias Papanikolaou, Senior Registrar)
- 1<sup>st</sup> University Department of Internal Medicine, General Hospital of Athens LAIKO, (PI: Michael Samarkos, Associate Professor of Internal Medicine and Infectious Diseases)
- 1<sup>st</sup> Department of Internal Medicine, General Hospital of Athens G. GENNIMATAS, (PI: Georgios Adamis, Director of NHS)
- 1<sup>st</sup> Department of Internal Medicine, General University Hospital of Ioannina, (PI: Charalampos Milionis, Professor of Internal Medicine)
- Department of Internal Medicine, General Hospital of Katerini (PI: Iraklis Tsanikidis, Director of NHS)

suPAR-GUIDED ANAKINRA TREATMENT FOR VALIDATION OF THE RISK AND EARLY MANAGEMENT OF SEVERE RESPIRATORY FAILURE BY COVID-19: THE SAVE OPEN-LABEL, NON-RANDOMIZED SINGLE-ARM TRIAL

- 1<sup>st</sup> Department of Internal Medicine, General Hospital of Athens KORGIALENIO-BENAKIO E.E.S. (PI: Vasiliki Tzavara, Director of NHS)
- 1<sup>st</sup> University Department of Pulmonary Medicine, SOTIRIA General Hospital of Chest Diseases of Athens (PI: Professor Antonia Koutsoukou)
- 5<sup>th</sup> Department of Pulmonary Medicine, SOTIRIA General Hospital of Chest Diseases of Athens (PI: Dimakou Aikaterini, Director of NHS)
- 1<sup>st</sup> Department of Internal Medicine, General Hospital of Nea Ionia CONSTANTOPOULIO-PATISION (PI: Masgala Aikaterini, Director of NHS)
- 1<sup>st</sup> Department of Internal Medicine, General Hospital of Eleusis THRIASIO (PI: Symbardi Styliani, Director of NHS)
- 2<sup>nd</sup> Department of Internal Medicine, General Hospital of Eleusis THRIASIO (PI: Alexiou Zoi, Director of NHS)
- Department of Internal Medicine, General Hospital of Athens ELPIS (PI: Fragkou Archontoula, Director of NHS)
- 3<sup>rd</sup> Department of Internal Medicine, General Hospital of Athens KORGIALENEIO-BENAKEIO E.E.S. (PI: Chini Maria, Director of NHS)
- 1<sup>st</sup> Department of Internal Medicine, General Hospital of Voula ASKLEPIEIO (PI: Bliziotis Ioannis, Registrar)
- 2<sup>nd</sup> Department of Internal Medicine, General Hospital of Piraeus TZANEIO (PI: Chrysos Georgios, Director of NHS)
- Department of Internal Medicine, General Hospital of Larissa KOUTLIMBANEIO & TPIANTAFYLLEIO (PI: Karakoussis Konstantinos, Director of NHS)
- 2<sup>nd</sup> Department of Internal Medicine, 251 Air Force General Hospital (PI: Symeonidis Nikolaos, Director)
- 1<sup>st</sup> Department of Internal Medicine, Prefecture General Hospital AMALIA FLEMING (PI: Georgios Marathonitis, Director of NHS)

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**APPENDIX II Study visits**

<b>Visit days</b>	<b>Screening</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>14</b>	<b>30</b>	<b>90</b>
Informed consent	x													
Exclusion criteria	x													
Inclusion criteria	x													
suPAR measurement	x							x						
Scoring of symptoms		x						x				x		
SOFA score		x						x				x		
Blood sampling		x						x						
Upper airways sampling		x							x					
Anakinra administration		x	x	x	x	x	x	x	x	x	x			
Severe respiratory failure			x	x	x	x	x	x	x	x	x	x		
AE/SAE		x	x	x	x	x	x	x	x	x	x	x		
Survival													x	x

**APPENDIX III The SOFA score**

Variable	0 points	1 point	2 points	3 points	4 points
PaO <sub>2</sub> /FiO <sub>2</sub> (mmHg)	≥400	<400	<300	<200	<100
Platelets (per mm <sup>3</sup> )	≥150	<150	<100	<50	<20
Hypotension	MAP≥ 70 mmHg	MAP<70 mmHg	Dobutamine whatever dose	Adrenaline≤0.1* or Noradrenaline ≤0.1*	Adrenaline>0.1* or Noradrenaline >0.1*
Glasgow Coma Scale	15	13-14	10-12	6-9	<6
Bilirubin (mg/dl)	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	≥12
Creatinine (mg/dl) or Urine output	<1.2	1.2-1.9	2.0-3.4	35-4.9 or <500ml/day	≥5.0 or <200ml/day

\*µg/kg/min

Each variable is scored between 0 and 4. The SOFA score is the sum of the score of each variable

**APPENDIX IV Scoring of respiratory symptoms**

<b>Symptom</b>	<b>Absent (score=0)</b>	<b>Mild (score=1)</b>	<b>Moderate (score=2)</b>	<b>Severe (score=3)</b>
<b>Cough</b>	No cough or resolution (to pre-CAP levels)	Cough present but it does not interfere with subject's usual daily activities	Cough present, frequent and it does interfere with some of the subject's usual daily activities	Cough is present throughout the day and night; it limits most of the subject's usual daily activities and sleep patterns
<b>Chest pain</b>	No chest pain or resolution of chest pain related to CAP	Chest pain present occasionally with deep breathing but it does not interfere with subject's usual daily activities	Chest pain is present with normal breaths and it does interfere with the subject's usual daily activities	Chest pain is present at rest and/or with shallow breathing; it limits most of the subject's usual daily activities
<b>Shortness of breath (dyspnea)</b>	No shortness of breath or resolution (to pre-CAP Baseline)	Shortness of breath with strenuous activities only but it does not interfere with subject's usual daily activities	Shortness of breath with usual activities and it does interfere with the subject's usual daily activities	Shortness of breath with minimal exertion or at rest; it limits most of the subject's usual daily activities
<b>Sputum</b>	No coughing up of phlegm/sputum or resolution (to pre-CAP Baseline)	Subject coughs up a small amount of phlegm/sputum	Subject coughs up a moderate amount of phlegm/sputum	Subject coughs up a large amount of phlegm/sputum

## **APPENDIX V Definition of severe respiratory failure**

Presence of all of the following:

- $pO_2/FiO_2$  less than 150
- Need for mechanical or non-mechanical ventilation (CPAP)