



Anakinra Efficacy in COVID-19 Pneumonia Guided by Soluble Urokinase Plasminogen Activator Receptor: Association With the Inflammatory Burden of the Host

Evdoxia Kyriazopoulou^a, Karolina Akinosoglou^b, Eleni Florou^c, Elli Kouriannidi^a, Artemis Bogosian^c, Olga Tsachouridou^d, Konstantinos N. Syrigos^e, Nikolaos Gatselis^f, Haralampos Milionis^g, Ilias C. Papanikolaou^h, Styliani Sympardiⁱ, Maria Dafni^j, Antonia Alevizou^a, Alexia-Vasiliki Amvrazi^a, Errika Alexandrou^a, Kyprianos Archontoulis^a, Katerina Argyraki^k, Zoi Alexiou^l, Yakinthi Georgiou^a, Dimitra Gkogka^a, Foteini Kyrailidi^a, Vassiliki Kalyva^a, Triantafilli Nikolopoulou^m, Sofia Ioannouⁿ, Petros Bakakos^o, Georgia Karathanassiou^a, Kyriakos Koklanos^a, Dionysios-Nikolaos Miletis^a, Anna-Maria Tili^a, Lampros Vakkas^a, Ioanna Vila^a, Periklis Panagopoulos^p, Michael Samarkos^q, George Chrysos^r, George N. Dalekos^f, Garyphallia Poulakou^e, Symeon Metallidis^d, Evangelos J. Giamarellos-Bourboulis^{a,c,*}

^a Fourth Department of Internal Medicine, National and Kapodistrian University of Athens, Medical School, Athens, Greece

^b Department of Internal Medicine, University of Patras, Rion, Greece

^c Hellenic Institute for the Study of Sepsis, Athens, Greece

^d First Department of Internal Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

^e Third Department of Internal Medicine, National and Kapodistrian University of Athens, Medical School, Athens, Greece

^f Department of Medicine and Research Laboratory of Internal Medicine, National Expertise Center of Greece in Autoimmune Liver Diseases, Full Member of the European Reference Network on Hepatological Diseases (ERN RARE-LIVER), General University Hospital of Larissa, Larissa, Greece

^g First Department of Internal Medicine, University of Ioannina, Medical School, Ioannina, Greece

^h Department of Pulmonary Medicine, General Hospital of Corfu "Agia Eirini", Greece

ⁱ First Department of Internal Medicine, Thriasio General Hospital of Eleusis, Athens, Greece

^j First Department of Internal Medicine, Korgialeneion-Benakeion General Hospital, Athens, Greece

^k Department of Internal Medicine, Sotiria Athens Hospital of Chest Diseases, Athens, Greece

^l Second Department of Internal Medicine, Thriasio General Hospital of Eleusis, Athens, Greece

^m First Department of Internal Medicine, G. Gennimatas General Hospital of Athens, Athens, Greece

ⁿ Department of Therapeutics, National and Kapodistrian University of Athens, Athens, Greece

^o First Department of Pulmonary Medicine, National and Kapodistrian University of Athens, Athens, Greece

^p Second Department of Internal Medicine, Democritus University of Thrace, Alexandroupolis, Greece

^q First Department of Internal Medicine, National and Kapodistrian University of Athens, Athens, Greece

^r Second Department of Internal Medicine, Tzaneio General Hospital of Piraeus, Athens, Greece

ARTICLE INFO

Article history:

Received 10 April 2024

Accepted 3 December 2024

Editor: A Çağkan İnkaya

Keywords:

COVID-19

suPAR

Anakinra

Severe respiratory failure

Mortality

ABSTRACT

Background: Anakinra was approved by the European Medicines Agency and received Emergency Use Authorization by the United States Food and Drug Administration for patients with COVID-19 pneumonia at risk for severe respiratory failure (SRF) with blood levels of soluble urokinase plasminogen activator receptor (suPAR) ≥ 6 ng/mL. We report the final results of the phase II open-label single-arm SAVE trial in a large population.

Methods: Patients with COVID-19 pneumonia and suPAR levels ≥ 6 ng/mL received subcutaneous anakinra 100 mg once daily for 10 days. The primary outcome was the incidence of SRF by day 14. Secondary outcomes were 30-day mortality, incidence of SRF according to time delay for start of treatment, safety, and associations with the inflammatory burden of the host.

* Corresponding author: 4th Department of Internal Medicine, ATTIKON University Hospital, 1 Rimini Street, 124 62 Athens, Greece. (E. J. Giamarellos-Bourboulis).
E-mail address: egiamarel@med.uoa.gr (E.J. Giamarellos-Bourboulis).

Results: From March 2020 to March 2022, a total of 992 patients were enrolled. The incidence of SRF was 18.8%, similar to the results of the phase III pivotal SAVE-MORE trial. The overall 30-day mortality was 9.5%. Participants were divided into 4 subgroups according to time delay between symptoms onset and start of anakinra. The incidence of SRF was similar for all subgroups. Serious adverse events were reported in 15.4%; only 3 were possibly related to anakinra. The most common adverse event was increased liver function tests. A post hoc comparison with the pivotal phase III trial showed similar anakinra outcomes among patient subgroups by levels of inflammatory mediators and D-dimers.

Conclusions: Results support the efficacy of anakinra as being similar to that of the pivotal registrational trial for COVID-19 pneumonia. The lack of a comparator group is a limitation.

Trial Registration: ClinicalTrials.gov, NCT04357366

© 2024 The Author(s). Published by Elsevier Ltd.

This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)

Background

The pandemic by the novel coronavirus SARS-CoV-2 provided evidence on the clinical utility of precision immunotherapy. Anakinra, a recombinant human interleukin (IL)-1 receptor antagonist, was approved by the European Medicines Agency and received Emergency Use Authorization by the United States Food and Drug Administration (FDA) for patients with COVID-19 pneumonia who were at risk for progression into severe respiratory failure (SRF) [1,2]. In the drug labeling, "risk" is defined as blood levels of the biomarker soluble urokinase plasminogen activator receptor (suPAR) of 6 ng/mL or more, which indicates early activation of the IL-1 cascade. Drug approval is based on the favorable results of the pivotal phase III SAVE-MORE trial [3,4]. In this trial, anakinra treatment guided by suPAR showed 0.36 proportional odds for worse outcome after 28 days as well as a decrease in the incidence of SRF compared to those in patients treated with placebo. Because suPAR is not licensed in the United States, the FDA has suggested a score of 8 variables to replace suPAR; patients meeting 3 of the variables are eligible for anakinra treatment [2].

The first evidence for the efficacy of this precision strategy came by the interim analysis of a prospective phase II open-label trial, in which all participants with COVID-19 pneumonia and elevated suPAR received anakinra for 10 days. Comparisons were done with concurrent comparators of similar severity receiving standard-of-care (SoC) treatment [5]. At the interim analysis, we questioned whether treatment delay may have an impact on clinical outcome. For this purpose, the phase II study SAVE continued, and results on the finally enrolled larger study population are presented herein. Since the SAVE study did not have a comparator arm, outcomes were compared to those of participants in the pivotal SAVE-MORE randomized clinical trial (RCT).

Methods

Trial oversight

SAVE is a prospective open-label single-arm phase II interventional trial conducted in 17 study sites in Greece (Supplementary Table 1). The trial was approved by the National Ethics Committee (approval 38/20) and the National Organization for Medicines (approval ISO 28/20), and was registered before enrollment of the first patient in EudraCT and ClinicalTrials.gov trial databases (EudraCT number 2020-001466-11; ClinicalTrials.gov registration number NCT04357366). The trial was sponsored by the Hellenic Institute for the Study of Sepsis (HISS) and funded in part by HISS, by Technomar Shipping Inc, and by Swedish Orphan Biovitrum AB. The laboratory of Immunology of Infectious Diseases of the 4th Department of Internal Medicine at ATTIKON University General Hospital acted as the central laboratory [5].

Originally the sample size was calculated assuming that the incidence of SRF would decrease from 60% to 45% with anakinra treatment. However, when results of the interim analysis were available, it was deemed necessary to continue the study with the aim to investigate whether treatment delay may have an impact on the primary endpoint. An amendment of the trial was approved (version 3.0) by the National Ethics Committee of Greece and by the National Organization for Medicines of Greece for this predefined subgroup analysis. The amendment was 90% powered at the 5% level of significance for 1000 patients, with the assumption that the incidence of SRF would be different between patients starting anakinra with more than 2 days of delay from hospital admission (estimated SRF incidence 25%) and patients starting anakinra within 2 days from hospital admission (estimated SRF incidence 15%) by 10%. This assumption was based on the rationale that an earlier start of treatment may result in better outcomes, as this has been suggested for other anti-cytokine drugs [6].

Participants

Study participants were adults with confirmed COVID-19 infection by reverse transcription polymerase chain reaction (RT-PCR) for SARS-CoV-2, with radiological evidence of pneumonia, and with plasma suPAR 6 ng/mL or more measured by the suPARnostic® Quick Triage kit (Virogates S/A, Birkerød, Denmark). Patients with medical history of stage IV malignancy or primary immunodeficiency or receiving anti-cytokine biological treatment during the last month were excluded. Other exclusion criteria were as follows: neutropenia ($<1500/\text{mm}^3$); respiratory insufficiency requiring mechanical ventilation (MV) or non-invasive ventilation (NIV); corticosteroid treatment defined as $\geq 0.4\text{mg/kg/d}$ of equivalent prednisone the last 15 days; do not resuscitate decision; and pregnancy or lactation. Patients or legal representatives provided written informed consent before screening.

Procedures

Enrolled patients were treated with 100 mg anakinra subcutaneously once daily for 10 days and standard-of-care (SoC) treatment according to local and international guidelines for COVID-19 [7]. Other drugs were allowed at the discretion of the treating physicians. Baseline plasma concentrations of IL-6, (Invitrogen, Carlsbad, California, USA) and ferritin (ORGENTEC Diagnostika GmbH, Mainz, Germany) were measured in duplicate by an enzyme immunoassay. Baseline plasma concentrations of C-reactive protein (CRP) were measured using a nephelometric assay (Siemens, Forchheim, Germany) and of D-dimers using the Stratus® CS 200 Acute Care™ Diagnostic Analyzer (Siemens). The lowest detection limits were as follows: for IL-6, 10 pg/mL; for ferritin, 75 ng/mL; for CRP, 3 mg/L; and for D-dimers, 0.1 ng/mL. The COVID-ETF (Emergency Task Force) committee of the European

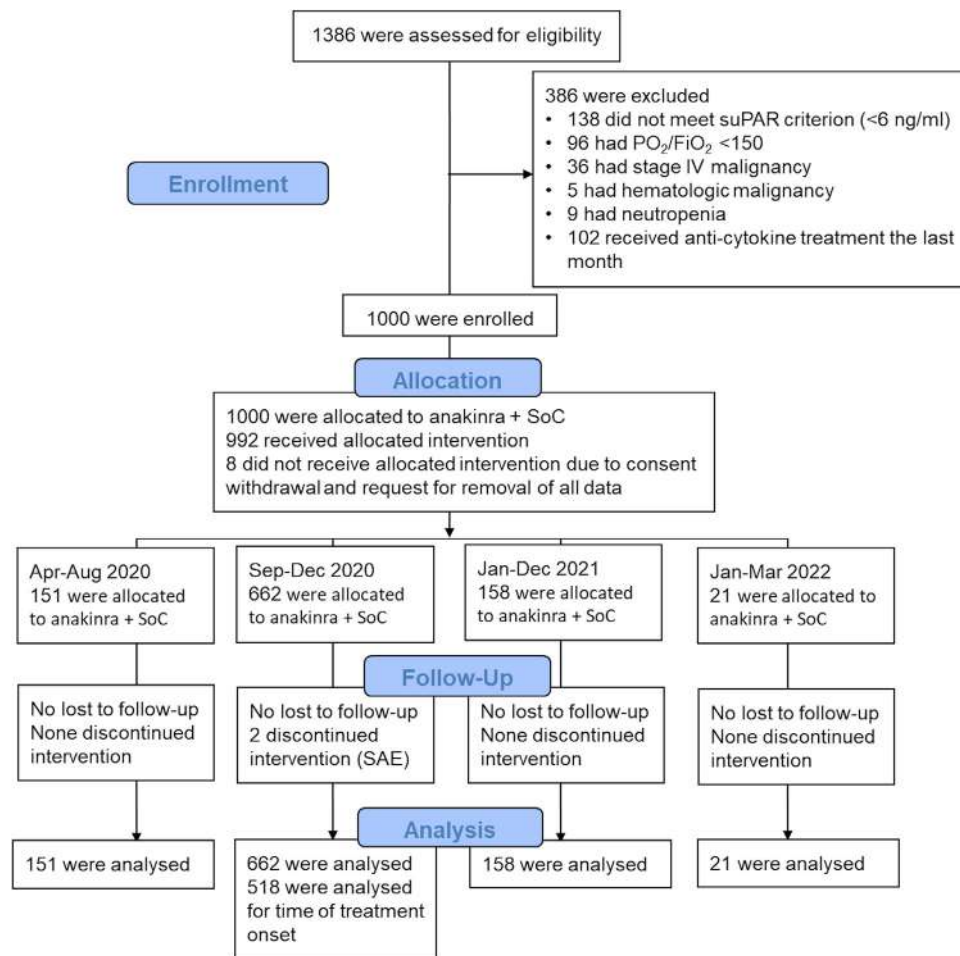


Figure 1. Study flow chart. SAE, serious adverse event; SoC, standard of care.

Medicines Agency advised measurement of these 4 biomarkers in patients in the SAVE-MORE trial [3]. This led us to select the same biomarkers for measurement in the SAVE trial, as these biomarkers also predict severity and unfavorable outcome of COVID-19 [8,9].

Outcomes

The primary endpoint was the incidence of SRF by day 14 after the start of the study drug. SRF was defined as any decrease of $\text{paO}_2/\text{FiO}_2$ below 150 requiring MV or NIV. If patients died before day 14, they were considered as meeting the primary endpoint. Secondary outcomes were 30-day mortality, 90-day mortality, intensive care unit (ICU) length of stay, change in sequential organ failure assessment (SOFA) score, the association of time delay from hospital admission until start of treatment with progression into SRF, the association of time delay from onset of symptoms until the start of treatment with progression into SRF, and the impact of comorbidities and period of hospitalization. The periods of hospitalization were selected to represent separate waves of the pandemic caused by different variants of SARS-CoV-2. The incidence of SRF in both arms of the SAVE-MORE trial was retrospectively compared to that of the SAVE trial. The incidence of SRF was a predefined secondary endpoint of the SAVE-MORE trial [3]. SRF in the SAVE-MORE trial was defined exactly as in the SAVE trial. Safety was assessed by reporting all non-serious treatment-emergent adverse events (TEAEs) and serious TEAEs (Common Terminology Criteria for Adverse Events, version 4.03).

Statistical analysis

Qualitative data were presented as percentages with confidence intervals (CI) and quantitative data as median with quartiles. We calculated the quartiles of distribution of Charlson Comorbidity Index, of time delay from symptoms onset until start of anakinra and of the $\text{paO}_2/\text{FiO}_2$ ratio at hospital admission. Comparisons were performed by forward stepwise Cox analysis. Comparisons with participants in the SAVE-MORE trial were done by Cox regression analysis. To minimize bias, demographic data, severity, and SoC treatment were compared between participants in the SAVE and SAVE-MORE trials. Comparisons were done using the χ^2 test for qualitative data, analysis of variance for quantitative data with normal distribution, and the Kruskal-Wallis test for quantitative data without normal distributions. Variables which were found to be different were analyzed as independent variables in a multivariate Cox regression model. Receiver operating characteristic (ROC) curves for IL-6, ferritin, CRP, and D-dimers were plotted to define the best cut-off for prediction of SRF. For ROC plotting, the total cohorts of SAVE and SAVE-MORE participants were used. The best cut-off was defined as the Youden index, which could discriminate between patients progressing into SRF and patients not progressing into SRF. Patients were then classified as presenting with increased values if above the cut-offs or with non-increased values if below the cut-offs. The progression to SRF was compared between participants in the SAVE and SAVE-MORE trials according to the presence of increase of any of the 4 biomarkers using logistic regression analysis. Any 2-sided P value < 0.05 was considered

Table 1
Baseline characteristics of enrolled patients (N = 992).

Characteristics	Values
Age, y, mean (SD)	62 (14)
Male sex, n (%)	586 (59.1)
Charlson Comorbidity Index, mean (SD)	2.4 (1.9)
SOFA score, mean (SD)	2.15 (1.36)
WHO classification for COVID-19, n (%)	
Moderate pneumonia	224 (22.6)
Severe pneumonia*	768 (77.4)
Days to start of study drug, median (Q1–Q3)	
From symptom onset	9 (6–11)
From hospital admission	1 (1–3)
Laboratory values, median (Q1–Q3)	
White blood cell count, cells per mm ³	5810 (4465–8215)
Lymphocyte count, cells per mm ³	920 (690–1260)
C-reactive protein, mg/L	40.6 (16.3–77.3)
Ferritin, ng/mL	401.0 (206.6–758.0)
Serum soluble uPAR, ng/mL	9.2 (7.4–11.9)
PaO ₂ : FiO ₂	282 (198–356)
Comorbidities, n (%)	
Type 2 diabetes mellitus	206 (20.8)
Chronic heart failure	43 (4.3)
Chronic renal disease	25 (2.5)
Chronic obstructive pulmonary disease	55 (5.6)
Coronary heart disease	85 (8.6)
Co-administered medications, n (%)	
Remdesivir	291 (29.3)
Dexamethasone	544 (54.8)
Combination of β -lactams with β -lactamase inhibitors	279 (28.1)
Piperacillin/tazobactam	127 (12.8)
Ceftriaxone	413 (41.6)
Ceftaroline	165 (16.7)
Respiratory fluoroquinolone	96 (9.7)
Azithromycin	375 (37.8)
Hydroxychloroquine	90 (9.1)

Abbreviations: FiO₂, fraction of inspired oxygen; PaO₂, partial oxygen pressure; SD, standard deviation; SOFA, sequential organ failure assessment; Q, quartile; WHO, World Health Organization.

* Defined as oxygen saturation less than 90% or more than 30 breaths/min or signs of respiratory distress.

statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software version 29.0.

Results

Patients

From March 2020 to March 2022, a total of 1,000 patients were enrolled; 8 patients withdrew consent and requested removal of all data, leaving a final intention-to-treat (ITT) analysis cohort of 992 patients (Figure 1). For 2 patients, the information of time since onset of symptoms until the start of anakinra and of time since hospital admission until the start of anakinra was not captured. These patients were not analyzed for the secondary endpoints of the impact of time delays from the start of symptoms and of hospital admission until the start of the study drug. Baseline characteristics of all enrolled patients are presented in Table 1.

Outcomes

A total of 186 patients (18.8%, 95% CI, 16.4–21.3%) among the ITT population progressed into SRF until day 14 (Table 2). Mortality after 30 days was 9.5% (95% CI, 7.8–11.5%) and after 90 days 12.2% (95% CI, 10.3–14.4%) (Table 2). A mean decrease of 1 (\pm 2) point of SOFA score was achieved under treatment with anakinra at day 14.

At the first wave of the pandemic, dexamethasone was not introduced in the SoC by the World Health Organization (WHO).

Dexamethasone was introduced in the SoC during the Delta variant–prominent second wave. The incidence of SRF and 30-day mortality were similar between the 2 waves. SRF was 21.2% (95% CI, 15.4–28.4%) during the first wave and 21.7% (95% CI, 18.7–25.0%) during the second wave. Mortality by day 30 was 10.6% (95% CI, 6.6–16.5%) and 11.5% (95% CI, 9.3–14.2%), respectively. Patients who died were mainly male with severe pneumonia (Supplementary Table 2). The incidence of SRF (6.3% in the third and 0% in the fourth period, respectively) and mortality (1.3% in the third and 0% in the fourth period, respectively) declined significantly in the following waves characterized by prevailing of the Omicron variant of SARS-CoV-2.

The incidence of SRF among anakinra-treated patients was lower (7.3%, 95% CI, 4.2–12.3%) among patients without any comorbidity (age-adjusted Charlson Comorbidity Index [CCI]=0) and was similar among patients with comorbidities (Supplementary Figure 1A). Similarly, the incidence of SRF was lower (2.3%, 95% CI, 0.9–5.85%) among patients at the upper quartile of the paO₂/FiO₂ ratio (Supplementary Figure 1B).

A total of 581 patients started anakinra the first 2 days from hospital admission; 98 patients (16.9%) progressed into SRF. The incidence of SRF was 14.2% (n = 38) for 267 patients who started anakinra more than 2 days after hospital admission (Figure 2A). Their baseline characteristics were similar (Supplementary Table 3). The incidence of SRF did not differ by the time delay of start of treatment since the onset of symptoms (Figure 2B and Supplementary Table 4). suPAR levels were similar in all patients irrespective of time delay from first symptoms of COVID-19 pneumonia until the start of the study drug, in contrast to other inflammatory

Table 2
Primary and secondary study endpoints (N = 992).

Outcomes	Values
Development of severe respiratory failure by day 14, n (%)	186 (18.8)
Need for non-invasive ventilation by day 14, n (%)	70 (7.1)
Need for invasive mechanical ventilation by day 14, n (%)	125 (12.6)
Mortality at day 30, n (%)	94 (9.5)
Mortality at day 90, n (%)	121 (12.2)
Absolute decrease in SOFA score at day 7 from baseline day 1, median (IQR)	0 (1)
Absolute decrease in SOFA score at day 14 from baseline day 1, median (IQR)	1 (2)
Median (Q1, Q3) time to hospital discharge, days	11 (9–15)
Median (Q1, Q3) time of ICU stay, days*	13 (7–19)

Abbreviations; ICU, intensive care unit; IQR, interquartile range; SOFA, sequential organ failure assessment; Q, quartile.

* Only for patients admitted to the ICU.

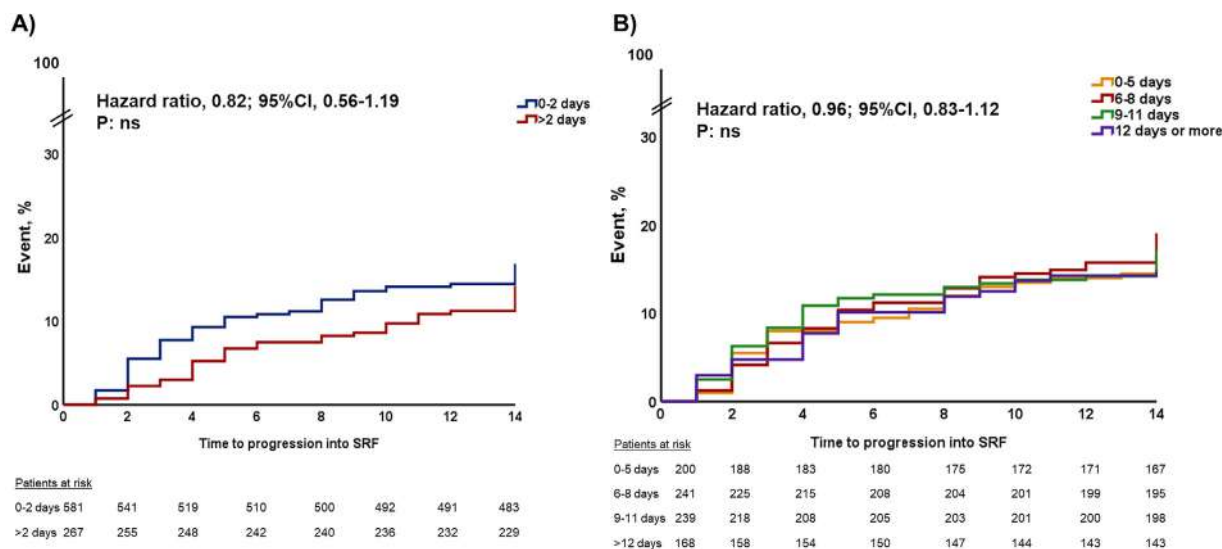


Figure 2. Time to progression into severe respiratory failure (SRF) according to time delay of start of anakinra. Patients are divided into subgroups of time delay from hospital admission until start of the study drug (A) and in subgroups of time delay from onset of symptoms until start of the study drug (B). CI, confidence interval; ns, non-significant.

biomarkers, such as IL-6, ferritin, and CRP (Supplementary Figure 2).

Exploratory analysis: comparison of SAVE study participants with the pivotal SAVE-MORE study

SAVE-MORE is the pivotal phase III RCT of the registration of anakinra for the treatment of COVID-19 pneumonia [1–3]. Baseline demographics of SAVE and SAVE-MORE participants were similar (Supplementary Table 5). The incidence of SRF was significantly decreased among participants in the SAVE trial treated with anakinra compared to placebo-treated participants in the SAVE-MORE trial even after adjustment for CRP and ferritin levels and severity of COVID-19 by WHO classification (adjusted hazard ratio [HR_{adj}], 0.77, 95% CI, 0.66–0.90; $P < 0.0001$) (Figure 3, Supplementary Table 6). The incidence of SRF was similar among participants in the SAVE trial treated with anakinra compared to anakinra-treated participants in the SAVE-MORE trial (Figure 3, Supplementary Table 6).

Exploratory analysis: anakinra efficacy by level of inflammation and coagulation

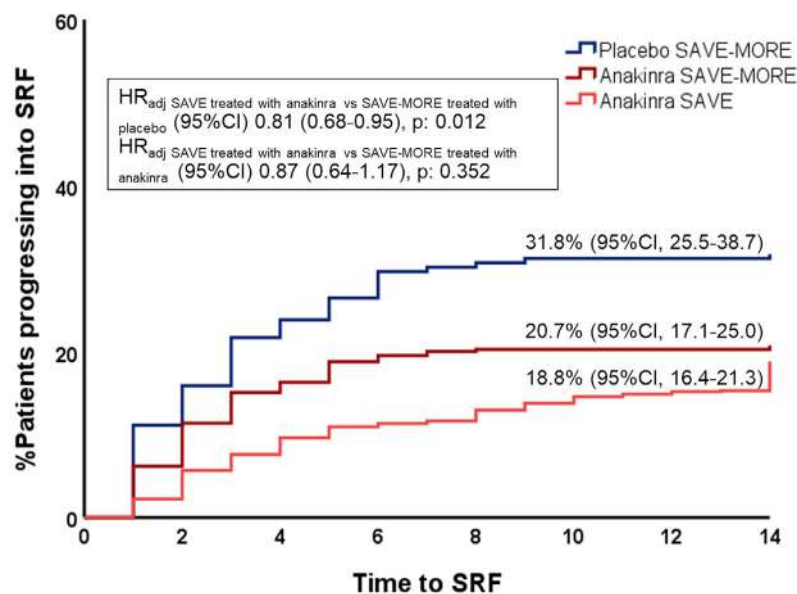
All 3 biomarkers were measured in 765 participants in the SAVE trial and 594 participants in the SAVE-MORE trial. The Youden index values of the respective ROC curves were 45 mg/L for CRP, 22

pg/mL for IL-6, and 680 ng/mL for ferritin. The progression into SRF was lower among participants in the SAVE trial treated with anakinra compared to participants in the SAVE-MORE trial treated with placebo when levels of these inflammation biomarkers were increased above the cut-offs (Figure 4A). No differences were found between participants in both trials treated with anakinra (Figure 4B).

The Youden index value of the ROC curve of D-dimers was 560 ng/mL. Among patients with D-dimers above these values, anakinra treatment decreased the incidence of SRF in SAVE patients compared to placebo-treated patients in the SAVE-MORE trial (Figure 4A). No difference was found between participants in both trials treated with anakinra (Figure 4B).

Safety

A total of 152 patients (15.3%) experienced at least 1 serious TEAE; only 3 out of a total of 205 serious TEAEs were considered by the investigators as possibly related to the study drug. These TEAEs were increase in liver function test results; in 2 cases, the study drug was stopped prematurely. At least 1 non-serious TEAE was experienced by 442 patients (44.6%). The most common non-serious TEAEs were increase in liver function test results and hypokalemia. Neutropenia was reported in 32 patients (3.2%); no patient had Grade 3 neutropenia (Table 3).



Patients at risk								
Placebo SAVE-MORE	189	159	144	133	131	130	130	129
Anakinra SAVE-MORE	405	359	339	326	323	323	323	321
Anakinra SAVE	992	936	897	880	864	848	842	806

Figure 3. Exploratory analysis of the progression into severe respiratory failure (SRF) of participants in the SAVE and SAVE-MORE trials. SAVE is an open-label trial, and all study participants were treated with anakinra. SAVE-MORE is a pivotal phase III randomized controlled trial comparing the efficacy of placebo versus anakinra adjunctive treatment to standard-of-care treatment. *P* values for comparisons by Cox regression analysis are provided after adjustment for severity of COVID-19 pneumonia by World Health Organization classification. CI, confidence interval; HR, hazard ratio.

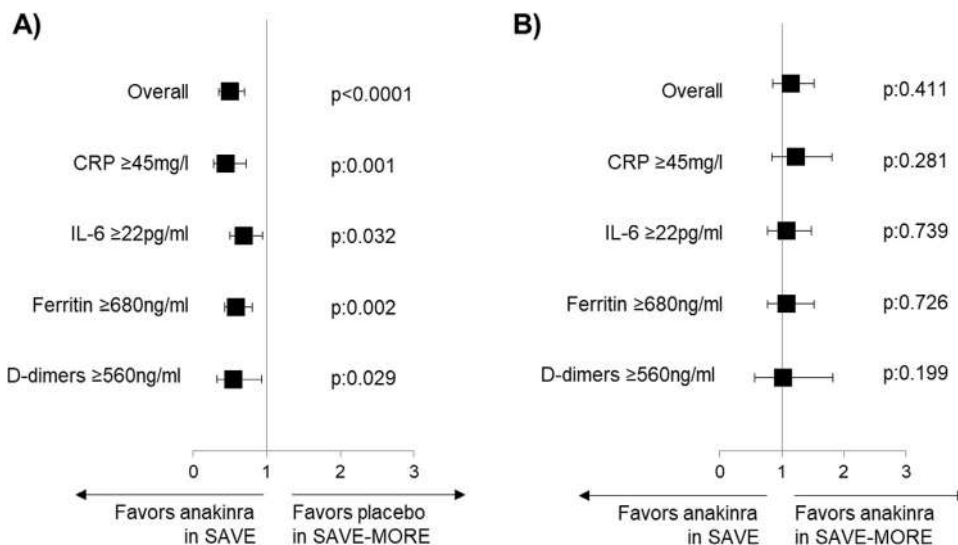


Figure 4. Exploratory analysis of the progression into severe respiratory failure (SRF) in relation to the degree of inflammation and coagulation between participants in the SAVE and SAVE-MORE trials. SAVE is an open-label trial and all study participants were treated with anakinra. SAVE-MORE is a pivotal phase III randomized controlled trial comparing the efficacy of placebo versus anakinra adjunctive treatment to standard-of-care treatment. (A) Odds ratios for the incidence of SRF among patients subgrouped by the blood levels of CRP, IL-6, ferritin, and D-dimers before start of treatment between participants in the SAVE trial treated with anakinra and participants in the SAVE-MORE trial treated with placebo. (B) Odds ratios for the incidence of SRF among patients subgrouped by blood levels of CRP, IL-6, ferritin, and D-dimers before start of treatment between participants in the SAVE trial treated with anakinra and participants in the SAVE-MORE trial treated with anakinra. CI, confidence interval; CRP, C-reactive protein; IL, interleukin; OR, odds ratio.

Discussion

In the SAVE study, 18.8% of COVID-19 patients admitted to the hospital with pneumonia and suPAR ≥ 6 ng/mL progressed into SRF with a 9.5% 30-day mortality. The incidence of SRF was similar irrespective of the delay from the onset of symptoms until the start of anakinra. Anakinra treatment did not raise safety concerns.

One post hoc analysis including participants in the SAVE-MORE pivotal trial showed that participants in the SAVE trial treated with anakinra has outcomes similar to those of participants in the SAVE-MORE trial treated with anakinra. The large patient number reaching almost 1000 patients providing both efficacy and safety data and the subgroup analysis towards the level of inflammation and coagulation make the novel contribution of this report.

Table 3
Most common (>2%) serious and non-serious treatment-emergent adverse events (TEAEs).

TEAEs	Values
At least 1 serious TEAE, n (%)	152 (15.4)*
Type of serious TEAE, n (%)	
Hospital-acquired pneumonia	72 (7.3)
Bloodstream infection	8 (0.8)
Acute pyelonephritis	4 (0.4)
Intraabdominal infection	1 (0.1)
<i>Clostridioides difficile</i> infection	4 (0.4)
Pulmonary embolism	12 (1.2)
Shock	68 (6.9)
Acute kidney injury	51 (5.2)
Increase in liver function test results	4 (0.4)
Thrombopenia	1 (0.1)
At least 1 non-serious TEAE, n (%)	442 (44.6)
Type of TEAE, n (%)	
Neutropenia	32 (3.2)
Grade 3	0 (0.0)
Anemia	76 (7.7)
Grade 3	2 (0.2)
Thrombocytopenia	50 (5.1)
Grade 3	3 (0.3)
Diarrhea	74 (7.5)
Grade 3	1 (0.1)
Increase in liver function test results	228 (23.0)
Grade 3	7 (0.7)
Bradycardia	43 (4.3)
Grade 3	2 (0.2)
Hyperkalaemia	23 (2.3)
Grade 3	2 (0.2)
Hypokalaemia	197 (19.9)
Grade 3	4 (0.4)

* 3 were judged as possibly related to study-drug (all cases concerned increase of liver function tests).

The SAVE study emphasizes the feasibility of using the predictive biomarker suPAR to identify individuals who would benefit from anakinra treatment. This marker has previously demonstrated its ability to predict unfavorable outcomes in COVID-19 and sepsis [10–13]. During the first few months since the start of the SAVE trial, several small-scale studies highlighted the positive effects of anakinra treatment in COVID-19 pneumonia [5,14–18]. In a real-world study, patients treated with anakinra guided by suPAR were protected from unfavorable outcomes [19]. Meta-analyses including mainly data from observational studies showed a decreased risk of death with anakinra treatment [20–22]. Existing RCTs differ in the size and the disease severity of participants; they also do not use any biomarker of predictive enrichment. Three meta-analyses including RCTs have been published: 2 integrated the results of 5 RCTs, and the third integrated the results of 7 RCTs [23–25]. The authors of these meta-analyses concluded that anakinra treatment was not associated with reduced mortality in hospitalized patients with COVID-19. However, the third meta-analysis showed that anakinra treatment reduced the need for MV in hospitalized patients [26].

Two main limitations of the SAVE trial need to be acknowledged. The first limitation is the single-arm open-label design. This limitation was known from the start of the trial in April 2020. At those times, the pressure for treatments worldwide questioned the need for placebo-treated comparators and guided the single-arm design. In order to minimize this limitation, comparisons with the placebo arm of the SAVE-MORE pivotal clinical trial were done. The second limitation was the lack of randomization for delay from the onset of symptoms or from hospital admission. However, the analysis of the randomized SAVE-MORE trial revealed the lack of impact of this time delay on anakinra efficacy [4]. suPAR indicates high blood levels of S100A8/A9 (calprotectin) in COVID-19 pneumonia. Animal challenge with plasma collected from patients

before progression into SRF stimulated high pro-inflammatory responses in the lung and colon. Pre-treatment with antibodies selectively targeting S100A8/A9 and murine IL-1 α prevented exacerbated inflammation of the lung and colon. This led to the consideration that suPAR is a biomarker of released danger-associated molecular patterns which stimulate the IL-1 cascade [27].

It needs to be emphasized that the efficacy of anakinra for COVID-19 pneumonia cannot be extrapolated to bacterial sepsis, as previously stated elsewhere [28]. The synergy of anakinra with corticosteroids in COVID-19 may be explained by the targeted mechanism of action of anakinra, which further enhances the non-specific anti-inflammatory effect of dexamethasone. It should be noted that tocilizumab providing benefit for severe and critical COVID-19 pneumonia was also administered as adjuvant to SoC dexamethasone [29].

The SAVE and SAVE-MORE trials make the vision of precision immunotherapy strategy for severe infections a reality. The central idea is to identify the activation of the IL-1 cascade and to strike at this window of opportunity. The time window is defined by the increase of the biomarker suPAR before clinical deterioration emerges. The similar outcomes from anakinra treatment in patients with both increased levels of inflammation and coagulation indicate that suPAR may be conceived as a biomarker of thromboinflammation. suPAR was increased in all patients irrespective the time delay since the onset of symptoms. These patients responded equally to anakinra treatment. The observations indicate that, in a precision strategy, the time from the onset of symptoms cannot predict the efficacy of treatment, and that the presence of the biomarker is the dominant factor which should guide treatment. The lack of widespread availability of suPAR in the United States limits the use of anakinra. However, the FDA has recently suggested a score from 8 variables (age, SOFA score, pneumonia severity, renal dysfunction, history of stroke, decreased haemoglobin, increased neutrophil/lymphocyte ratio, and smoking) as an alternative [2]. Results of the SAVE trial support the introduction of appropriate patient selection using biomarkers. The lack of a comparator group is acknowledged as a major limitation.

Declarations

Funding: This study was funded in part by the Hellenic Institute for the Study of Sepsis, in part by Technomar Shipping Company, and in part by Swedish Orphan BioVitrum AB (publ) (Sobi). Sobi provided financial support to the study and anakinra (Kineret®) free of charge under an Investigator Initiated Study Agreement. Sobi was not involved in the design or conduct of the study, data collection and analysis, or preparation of the manuscript.

Competing Interests: KA reports receiving honoraria and consulting fees from healthcare companies, including Angelini, MSD, Pfizer, Swedish Orphan Biotrivum AB, 3M hellas, GSK/ViiV, and Gilead. GP has received honoraria and/or consulting fees by AstraZeneca, Gilead, GSK, Menarini, MSD, Norma, Pfizer, and SOBI and research grants by the University of Minnesota/University College London, the Hellenic Institute for the Study of Sepsis, Bausch, Roche, Xenothera, FabNTEch, and Pfizer. HM reports receiving honoraria, consulting fees and non-financial support from healthcare companies, including Amgen, Angelini, Bayer, Mylan, MSD, Pfizer, and Servier. ICP received research funds and honoraria from AstraZeneca, Boehringer-Ingelheim, and Chiesi pharmaceuticals. GND has received research grants from Gilead and has served as advisor/lecturer for Ipsen, Pfizer, Genkyotex, Sanofi, Sobi and as PI for Amyndas Pharmaceuticals, Intercept Pharma, CymaBay Therapeutics, Genkyotex, Novo Nordisk, Pfizer, Regulus Therapeutics, Sobi, and Tiziana Life Sciences. EJGB has received honoraria from Abbott Products Operations, bioMérieux Inc, ThermoFisher Brahm GmbH,

GSK, InflaRx GmbH, Sobi, and Xbiotech Inc; independent educational grants from AbbVie, Abbott Products Operations, bioMérieux Inc, InflaRx GmbH, Johnson & Johnson, MSD, UCB, Sanofi, and Sobi.; and funding from the Horizon 2020 Marie Skłodowska-Curie International Training Network “the European Sepsis Academy” (granted to the National and Kapodistrian University of Athens), the Horizon 2020 European Grants ImmunoSep and RISCinCOVID and the Horizon Europe grants EPIC-CROWN-2, POINT, and Homi-Lung (granted to the Hellenic Institute for the Study of Sepsis). The other authors declare no conflicts of interest.

Ethical Approval: The trial was approved by the National Ethics Committee (approval 38/20) and the National Organization for Medicines (approval ISO 28/20) and was registered before patient enrollment.

Sequence Information: Not applicable.

Author contributions: EK performed data analysis and drafted the manuscript. KA drafted the manuscript. EIGB conceptualized the study, performed data analysis and revised the manuscript for critical content. EF, EK, AB, OT, KNS, NG, HM, ICP, SS, MD, AA, AVA, EA, KyA, KA, ZA, YG, DG, FK, VK, TN, SI, PB, GK, KK, DNM, AMT, LV, IV, PP, MS, GC, GND, GP, SM collected clinical data and revised the manuscript for critical content. All authors approved the version to be published.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.ijantimicag.2024.107405](https://doi.org/10.1016/j.ijantimicag.2024.107405).

References

- [1] European Medicines Agency. EMA recommends approval for use of Kineret in adults with COVID-19. 2021. <https://www.ema.europa.eu/en/news/ema-recommends-approval-use-kineret-adults-covid-19>.
- [2] Fact sheet for healthcare providers: emergency use authorization for Kineret <https://www.fda.gov/media/163075/download>.
- [3] Kyriazopoulou E, Poulakou G, Milionis H, Metallidis S, Adamis G, Tsiakos K, et al. Early treatment of COVID-19 with anakinra guided by soluble urokinase plasminogen receptor plasma levels: a double-blind, randomized controlled phase 3 trial. *Nat Med* 2021;27:1752–60.
- [4] Akinosoglou K, Kotsaki A, Gounaridi IM, Christaki E, Metallidis S, Adamis G, et al. Efficacy and safety of early soluble urokinase plasminogen receptor plasma-guided anakinra treatment of COVID-19 pneumonia: a subgroup analysis of the SAVE-MORE randomised trial. *EclinicalMedicine* 2023;56:101785.
- [5] Kyriazopoulou E, Panagopoulos P, Metallidis S, Dalekos GN, Poulakou G, Gatselis N, et al. An open label trial of anakinra to prevent respiratory failure in COVID-19. *Elife* 2021;10:e66125.
- [6] Winthrop KL, Mariette X. To immunosuppress: whom, when and how? That is the question with COVID-19. *Ann Rheum Dis* 2020;79:1129–31.
- [7] <https://www.loimoxeis.gr/covid-19-info-banner/>
- [8] Yao Y, Chen W, Wu X, et al. Clinical characteristics of COVID-19 patients in three consecutive generations of spread in Zhejiang, China. *Clin Microbiol Infect* 2020;26:1380–5.
- [9] Cheng B, Hu J, Zuo X, et al. Predictors of progression from moderate to severe coronavirus disease 2019: a retrospective cohort. *Clin Microbiol Infect* 2020;26:1400–5.
- [10] Rovina N, Akinosoglou K, Eugen-Olsen J, Hayek S, Reiser J, Giamarellos-Bourboulis EJ. Soluble urokinase plasminogen activator receptor (suPAR) as an early predictor of severe respiratory failure in patients with COVID-19 pneumonia. *Crit Care* 2020;24:187.
- [11] Giamarellos-Bourboulis EJ, Norrby-Teglund A, Mylona V, Savva A, Tsangaris I, Dimopoulou I, et al. Risk assessment in sepsis: a new prognostication rule by APACHE II score and serum soluble urokinase plasminogen activator receptor. *Crit Care* 2012;16:R149.
- [12] Hayek SS, Leaf DE, Reiser J. Soluble urokinase receptor and acute kidney injury. *Reply. N Engl J Med* 2020;382:2167–8.
- [13] Azam TU, Shadid HR, Blakely P, O'Hayer P, Berlin H, Pan M, et al. Soluble urokinase receptor (SuPAR) in COVID-19-related AKI. *J Am Soc Nephrol* 2020;31:2725–35.
- [14] Cavalli G, Colafrancesco S, Emmi G, Imazio M, Lopalco G, Maggio MC, et al. Interleukin 1alpha: a comprehensive review on the role of IL-1alpha in the pathogenesis and treatment of autoimmune and inflammatory diseases. *Autoimmun Rev* 2021;20:102763.
- [15] Huet T, Beaussier H, Voisin O, Jouvessomme S, Dauriat G, Lazareth I, et al. Anakinra for severe forms of COVID-19: a cohort study. *Lancet Rheumatol* 2020;2:e393–400.
- [16] Cauchois R, Koubi M, Delarbre D, Manet C, Carvelli J, Blasco VB, et al. Early IL-1 receptor blockade in severe inflammatory respiratory failure complicating COVID-19. *Proc Natl Acad Sci U S A* 2020;117:18951–3.
- [17] Siyer O, Aksakal B, Basat S. Evaluation of the effects of anakinra treatment on clinic and laboratory results in patients with COVID-19. *North Clin Istanbul* 2023;10:189–96.
- [18] Al Kharusi M, Al Sheikh N, Alhajri M, Al Mandhri SA, Khafagy ES, Moglad EH, et al. A prospective cohort study of COVID-19: evaluation of the early role of IL-1 and IL-6 antagonists in improving the outcome of the illness and reduction in the risk of death. *Healthcare (Basel)* 2023;11. doi:10.3390/healthcare11071025.
- [19] Segala FV, Rando E, Salvati F, Negri M, Catania F, Sanmartin F, et al. Anakinra in hospitalized COVID-19 patients guided by baseline soluble urokinase plasminogen receptor plasma levels: a real world, retrospective cohort study. *PLoS One* 2023;18:e0273202.
- [20] Kyriazopoulou E, Huet T, Cavalli G, Gori A, Kyrianiou M, Pickkers P, et al. Effect of anakinra on mortality in patients with COVID-19: a systematic review and patient-level meta-analysis. *Lancet Rheumatol* 2021;3:e690–7.
- [21] Kyriakoulis KG, Kollias A, Poulakou G, Kyriakoulis IG, Trontzas IP, Charpidou A, et al. The effect of anakinra in hospitalized patients with COVID-19: an updated systematic review and meta-analysis. *J Clin Med* 2021;10. doi:10.3390/jcm10194462.
- [22] Barkas F, Filippas-Ntekouan S, Kosmidou M, Liberopoulos E, Liontos A, Milionis H. Anakinra in hospitalized non-intubated patients with coronavirus disease 2019: a systematic review and meta-analysis. *Rheumatology (Oxford)* 2021;60:5527–37.
- [23] Somagutta MKR, Lourdes Pormento MK, Hamid P, Hamdan A, Khan MA, Desir R, et al. The safety and efficacy of anakinra, an interleukin-1 antagonist in severe cases of covid-19: a systematic review and meta-analysis. *Infect Chemother* 2021;53:221–37.
- [24] Shang W, Zhang Y, Wang G, Han D. Anakinra was not associated with lower mortality in hospitalised COVID-19 patients: a systematic review and meta-analysis of randomized controlled trials. *Rev Med Virol* 2023;33:e2418.
- [25] Dahms K, Mikolajewska A, Ansems K, Metzendorf MI, Benstoem C, Stegemann M. Anakinra for the treatment of COVID-19 patients: a systematic review and meta-analysis. *Eur J Med Res* 2023;28:100.
- [26] Lan SH, Hsu CK, Chang SP, Lu LC, Lai CC. Clinical efficacy and safety of interleukin-1 blockade in the treatment of patients with COVID-19: a systematic review and meta-analysis of randomized controlled trials. *Ann Med* 2023;55:2208872.
- [27] Renieris G, Karakike E, Gkavogianni T, Droggiti DE, Stylianakis E, Andriopoulou T, et al. IL-1 mediates tissue-specific inflammation and severe respiratory failure in COVID-19. *J Inn Immun* 2022;14:643–56.
- [28] Shapiro L, Scherger S, Franco-Paredes C, Gharamti A, Henao-Martinez AF. Anakinra authorized to treat severe coronavirus disease 2019: sepsis breakthrough or time to reflect? *Front Microbiol* 2023;14:1250483.
- [29] RECOVERY Collaborative Group. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet* 2021;397:1637–45.