Efficacy and safety of asunercept, a CD95L-selective inhibitor, in hospitalised patients with moderate-to-severe COVID-19: ASUNCTIS, a multicentre, randomised, open-label, controlled, phase 2 trial

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Summary

Background The phase 2 ASUNCTIS study assessed the efficacy and safety of asunercept, a fully human CD95 (Fas) ligand-binding protein, in hospitalised patients with moderate-to-severe coronavirus disease (COVID-19) to assess the clinical benefit of CD95 ligand inhibition in this viral disease.

Methods In this open-label, multicentre, randomised, controlled, phase 2 trial, patients with COVID-19–induced pneumonia and respiratory deterioration were randomly assigned (1:1:1:1) in 12 Russian and Spanish hospitals using an interactive web-response system to receive standard of care (SOC) or SOC plus weekly asunercept 25 mg, 100 mg, or 400 mg, administered intravenously for up to 4 weeks, or until hospital discharge or death. The randomisation was stratified according to the respiratory support methods at the time of enrolment, corresponding to categories 4–6 of a clinical severity assessment scale comprising 9 levels that was recommended by the World Health Organization (WHO) at the time of the study. The main inclusion criterion was laboratory confirmed infection with SARS-CoV-2 OR typical radiological signs of SARS-CoV-2 infection. The primary endpoint was time from randomisation to clinical improvement on two consecutive days of at least one category on a WHO clinical severity assessment scale in the modified intent-to-treat population. All patients were subjected to regular safety analyses. This trial is registered with EudraCT (2020-001887-27) and ClinicalTrials.gov (NCT04535674).

Findings Between October 9, 2020, and September 24, 2021, 438 patients were randomly assigned to SOC (n = 110) or SOC plus asunercept 25 mg (n = 109), 100 mg (n = 109), or 400 mg (n = 110). The primary endpoint, time to

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sustained clinical improvement of one WHO category on two consecutive days from randomization, was in median [95% confidence interval]: 9 [6–12], 8 [7–12], 8 [7–11] and 13 [9–20] days for the 400 mg, 100 mg, 25 mg asunercept and SOC groups, respectively. The standard deviations for the 400 mg, 100 mg, 25 mg asunercept and SOC groups were 5.3, 4.9, 4.7 and 5 days, respectively. The observed differences between groups failed to reach statistical significance (one-sided p-value = 0.041). In total, 290 adverse events (AE) were registered in 145 patients who received at least one dose of the study treatment: 77 AEs in 37 (33.6%) patients in the SOC group, 80 AEs in 38 (34.9%) patients in the 25 mg group, 61 AEs in 35 (32.7%) patients in the 100 mg group and 72 AEs in 35 (32.1%) patients in the 400 mg group. There was no treatment-related death reported. In summary, asunercept was well tolerated at all doses tested and no specific safety signals were detected.

Interpretation The primary endpoint of time to sustained clinical improvement for distinct asunercept arms compared to SOC failed to meet statistical significance. The compound was safe and well tolerated.

Funding Apogenix GmbH, Heidelberg, Germany.

Keywords: Asunercept; CD95L (FasL); COVID-19; Phase 2 clinical trial; Lymphocytopenia; Apoptosis; Death ligand blockade

Research in context

Evidence before this study

Relevant publications supporting the potential efficacy of CD95L inhibition in patients with SARS-CoV-2 infection were identified by searching PubMed between March 28, 2020 and April 14, 2020, using search terms such as "COVID-19", "SARS-CoV-2", "CD95L/FasL", "apoptosis", and "ARDS/acute respiratory distress syndrome". Asunercept, a first-in-class CD95L blocker, has already been investigated in clinical trials in patients with cancer. Asunercept was safely administered to healthy volunteers and patients via the intravenous route in doses of up to 20 mg/kg (EudraCT No: 2008-000130-49). A controlled phase 2 clinical trial in patients with glioblastoma (n = 84) showed that asunercept was safe at a weekly dose of 400 mg when used in combination with radiotherapy and led to improved outcomes (NCT01071837). Furthermore, a phase 1/2 study (n = 29) provided clinical evidence that CD95L inhibition by asunercept protects erythrocyte precursor cells from undergoing apoptosis in patients with myelodysplastic syndromes, reducing the need for transfusions. The therapeutic efficacy of selective CD95L inhibition in individuals with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-induced pathology has not yet been tested in clinical studies.

Added value of this study

While the primary endpoint with respect to efficacy was not met with statistical significance, the results of the phase 2 ASUNCTIS study suggest that out of the three dose levels tested, 100 mg of asunercept per week may show the most favourable tendency for faster clinical improvements in patients who are hospitalised due to moderate-to-severe COVID-19 and require oxygen support, although these findings should be interpreted with caution. Importantly, asunercept treatment added to available standard of care (including corticosteroids, tocilizumab, remdesivir, and other antiviral medication) demonstrated good safety and tolerability.

Implications of all the available evidence

Asunercept represents an innovative approach to the treatment of hospitalised patients with moderate-to severe COVID-19. Positive trends in clinical efficacy have been observed. Based on this as well as on the excellent safety profile of the compound, further clinical investigations are warranted to examine therapeutic efficacy in larger patient populations similar to the one recruited in the ASUNCTIS study.

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a serious life-threatening disease that has affected approximately 775 million people worldwide and resulted in over 7 million deaths, as of March 2024.[1] Most individuals with COVID-19 will experience mild-to-moderate symptoms, but some patients, particularly those who are non-vaccinated, older, with comorbidities, and/or immunocompromised, may develop severe complications, including pneumonia, respiratory failure, or acute respiratory distress syndrome (ARDS),[2] the latter being associated with an intensive care unit (ICU) mortality rate of approximately 30–40%.[3] Especially worrisome are variants with increased transmissibility, vaccine escape and/or increased pathogenicity.[4]

CD95 ligand (CD95L or FasL) is a prototypic death ligand capable of inducing cellular apoptosis via engagement with the CD95 (Fas) death receptor. Soluble CD95L is found in the bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome (ARDS) and other types of lung injuries. Activation of CD95 signalling induces lung injury and fibrosis in preclinical models. Blockade of CD95L prevents microbial or haemorrhagic shock-induced lung failure in animals. Pharmacological inhibition of CD95L with CD95-Fc significantly reduces reo- and influenza virus-induced lethality in mice.

COVID-19 pathology is thought to result from a hyper-inflammatory response rather than a direct effect of virus-induced cell death.[5] Thus, there is an urgent need to understand and rationally target this immune dysregulation. A potential therapeutic target is the signalling pathway triggered by the interaction between the death ligand CD95 ligand (CD95L, also known as FasL) and its receptor, CD95 (also known as Fas or APO-1). An increase of CD95L in the bronchoalveolar lavages of patients with ARDS is associated with dismal prognosis.[6] One of the best understood physiological roles of CD95L is to maintain homeostasis of immune cells by induction of apoptotic cell death. CD95L has also been demonstrated to be highly relevant in the mediation of activation-induced cell death (AICD) in T cells.[7] Kreutmair and colleagues[8] proposed that CD95 over-activation is involved in the development of peripheral lymphocytopenia in COVID-19. Patients with lymphocytopenia tend to have a poorer disease prognosis than their counterparts.[9] Further, CD95L can kill lung epithelial cells[6,10] and exogenous CD95L can induce ARDS in rabbits.[11] Mutated CD95L or its pharmacological inhibition reduces murine reo- and influenza virus-induced lung failure, respectively.[12,13] We recently demonstrated that therapeutic inhibition of CD95L with a murine ortholog of asunercept enhanced survival of young and aged mice infected with mouse-adapted SARS-CoV-2.[14] In this model, CD95L was highly expressed by natural killer cells and inflammatory monocytic macrophages in the lungs of infected mice, two cell types that have been proposed to promote disease severity. Accordingly, CD95L was also significantly elevated in the bronchoalveolar lavage fluid of critically-ill COVID-19 patients.[14] Thus, preclinical models in combination with clinical observations implicate CD95L as a crucial driver of COVID-19 disease severity and suggest that the therapeutic inhibition of CD95L has the potential to attenuate inflammation–induced lung pathology, including when triggered by SARS-CoV-2 or other viruses.

Asunercept (CD95-Fc; Apogenix GmbH, Heidelberg, Germany) consists of the extracellular domain of human CD95 fused to the Fc region of human immunoglobulin G1 (IgG1). The compound blocks CD95L, thereby preventing it from triggering CD95-dependent signalling. Asunercept has been shown to be well tolerated in humans.[15] Thus, we hypothesized that asunercept may be an effective treatment for moderate-to-severe COVID-19. As it targets virus-induced host pathology and not virus propagation itself, the compound might be efficacious independent of the virus sub-type.

The aim of this study (ASUNCTIS) was to assess the efficacy and safety of asunercept as a treatment for hospitalised patients with moderate-to-severe COVID-19 and to identify the most effective dose, thus providing clinical evidence to support the use of asunercept as a treatment for COVID-19.

Methods

Study design and participants

ASUNCTIS was a prospective, multicentre, randomised, open-label, active-controlled, phase 2 study conducted at sites in Spain (n = 6) and Russia (n = 6), and assessed the efficacy and safety of asunercept in patients who had been hospitalised due to COVID-19–induced pneumonia and had signs of respiratory deterioration. Patients were enrolled from the pool of patients in the participating hospitals if they had a laboratory-confirmed infection with SARS-CoV-2 or typical radiological signs of SARS-CoV-2–induced pneumonia (computed tomography [CT] scan or chest X-ray with pulmonary infiltrates; Supplementary Table S1), and if they agreed to participate in the trial.

The trial was conducted in accordance with the requirements for the conduct of clinical studies as provided in the European Union Directive 2001/20/EC, and was approved by respective competent authorities as well as by ethics committees, namely the Spanish Ethics Committee on Research involving Medicinal Products, Aragon (Comité Ético de Investigación Clínica de Aragón; approval number 37652), the Spanish Agency for Medicines and Health Products (Agencia Española del Medicamento y Productos Sanitarios; approval number PEI20-115), and the Ministry of Healthcare of the Russian Federation (approval number 368). The general guidelines indicated in the Declaration of Helsinki, and all applicable regulatory requirements in the countries in which the study was performed, were abided by. Written informed consent was obtained from all patients, or their relatives, prior to being included in the study. Cross-over was not allowed in order to allow for group-to-group comparisons. In total, 139 protocol deviations have been observed. The vast majority of them (n = 90) were due to fact that biosamples have not been stored appropriately. Analyses of biosamples are not subject of this manuscript. In addition, some measurements of vital signs or respiratory functions are missing (n = 26) but this does not influence the assessment of the primary endpoint. In addition, some follow-up visits have not been performed (n = 6) and there were some data like, e.g., WHO classifications,

incorrectly entered into the CRF (n = 17). Reasons for censoring patients are summarised on Supplementary Table S2. Self-reported sex referred to biological attributes and was categorized as female or male.

The study protocol is available in the Supplementary Methods.

Randomisation and masking

Patients were randomised 1:1:1:1 using an interactive web-response system to receive standard of care (SOC) alone (the control arm) or SOC plus asunercept (25 mg, 100 mg, or 400 mg). The randomisation was stratified according to the respiratory support methods at the time of enrolment, corresponding to categories 4–6 of a clinical severity assessment scale comprising 9 levels that was recommended by the World Health Organization (WHO) at the time of the study (Supplementary Table S3). Participants, study staff, and investigators were not masked to the study assignment. There was competitive enrolment, i.e., no restrictions on the number of patients per site were implemented. The randomisation list and sequence were generated by the responsible biostatistician at the contract research organisation whereas actual enrolment was performed by the site staff via the computer-based service, which assigned the treatment arms based on the established randomisation sequence. The biostatistician was involved in activities related to interim and final study reporting.

Study procedures and outcomes

Asunercept was administered weekly by intravenous infusion at one of three dose levels (25 mg, 100 mg, or 400 mg) for up to four doses or until hospital discharge or death, whichever occurred first. SOC comprised, as necessary, (i) ventilation and oxygen support such as nasal prongs/masks, high-flow oxygen therapy, non-invasive mechanical ventilation, invasive mechanical ventilation or extracorporeal membrane oxygenation [ECMO], (ii) vasopressor support, (iii) renal-replacement therapy, (iv) antimicrobial agents and/or (v) immunotherapy including glucocorticoids and tocilizumab. Other experimental anti-inflammatory substances targeting the immune system were not permitted as part of local SOC. Other treatments that patients were receiving for conditions prior to hospitalisation could be continued. Antiviral treatments could also be administered, provided their use was documented.

The primary study objective was to investigate the efficacy of asunercept in hospitalised patients with moderate-to-severe COVID-19. The primary endpoint was the time from randomisation to sustained improvement of at least one category on the WHO clinical severity assessment scale (Supplementary Table S3) on two consecutive days, measured until Day 29, hospital discharge or death, whichever occurred first. Patients were assessed at the individual sites by the treating site staff at the screening visit (Visit 0), at baseline (Visit 1), daily during hospitalisation (until Day 29 [Visit 5], hospital discharge, early termination or death [whichever occurred first]), and at the follow-up assessment (Visit 6, Day 36). If the patient was discharged from hospital before Day 29, the follow-up assessment could be performed by telephone call.

The secondary objectives included: (i) oxygenation, with the endpoints of oxygenation free days and the duration of new oxygen use during the study; (ii) ventilation, with the endpoints ventilator free days until day 29 and incidence and duration of new mechanical ventilation use during the trial; (iii) National Early Warning Score (NEWS) with the endpoint time to discharge or to a NEWS of $\leq$2 that was maintained for 24 h, whichever occurred first (as changes from baseline); (iv) hospitalisation, with the endpoints duration of hospitalisation and stay in the ICU (days); (v) ICU admission, with the endpoint proportion of patients admitted to the ICU until day 29; and (vi) mortality, with the endpoints of 15-day, 29-day, 60-day, and 90-day all-cause mortality.

Safety objectives were the cumulative incidence of adverse events (AEs) that were considered serious AEs (SAEs), and discontinuation or temporary suspension of therapy. AEs were coded according to Medical Dictionary for Regulatory Activities (MedDRA, version 25.0) and recorded daily. They were summarised by each study arm, and the characteristics of the AE were determined (seriousness, severity, relationship to the administered treatment, outcome, and implemented actions). Changes in laboratory, biochemical, and immunological parameters over time were also safety objectives, including differential blood counts. The frequency of deviations from reference values for laboratory data, vital signs, and physical examinations were recorded.

The effect of asunercept exposure on lymphocyte recovery was assessed in an exploratory analysis. For graphical representation, lymphocyte counts were grouped by day (as measurements were taken at variable time points in relation to the first asunercept dosing due to the circumstances of the pandemic) and a last observation carried forward and next observation carried backward procedure was applied for days with missing individual data to obtain a complete dataset. However, the missing values precluded a statistical analysis of raw data. Hence, an exploratory non-linear mixed effects modelling approach using the NONMEM 7.4.3 software was employed to correlate plasma concentrations of asunercept with the measured lymphocyte counts and simulate the model predictions. As no plasma concentrations for asunercept were measured in the ASUNCTIS study, a previously established pharmacokinetic model was used to predict individual asunercept plasma concentrations based on patient covariates. The pharmacokinetic data were obtained from patients with

glioblastoma treated with asunercept.[16] This model was then integrated with a semi-mechanistic lymphocyte count model and accounted for different covariates such as lymphocyte counts at the beginning of treatment and treatment with asunercept. Further details of the model are described in the Supplementary Material and Supplementary Figure S1.

Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) was established. The DSMB voting members consisted of three independent clinical experts/physicians experienced in therapeutic areas relevant to COVID-19 disease and clinical research. The DSMB used available safety data to determine possible safety signals and to decide on recommendations to the sponsor for the continuation of the study or individual arms as planned, modification of the eligibility criteria or protocol procedures to enhance patient safety.

Statistical analysis

The initial sample size calculation was based on the two-group comparison between each of the asunercept-treated and SOC arms. It was assumed that in total 160 clinical improvements would be necessary to ensure a power of 80% to detect an improvement of approximately 5 days in the primary endpoint of time to clinical improvement if the median time in the control arm was 14 days. Assuming approximately 25% and 10–15% of patients in the SOC and asunercept arms, respectively, would have no improvement during the follow-up of 28 days, 100 patients were planned to be included in each study arm.

The modified intent-to-treat (mITT) set (i.e., all patients who were randomised and received at least one dose of study medication) was considered the primary set for the efficacy analyses. For sensitivity analyses, primary and secondary endpoints were also analysed in the per-protocol (PP) set (i.e., all patients who received at least one dose of study medication and had no major protocol violations). The safety set included all patients who had received at least one dose of study treatment. The statistics calculated for the different types of variables are outlined in Supplementary Table S4.

For the primary analysis of time to improvement, patients without improvement were censored using the date of the first event (Day 29, hospital discharge or end of study). Patients who died were censored (i.e., no improvement) and contributed to the analysis with a follow-up time of the maximum value between study day of death and Day 28. Pairwise comparisons between treatment arms and control were performed using the log-rank test. The corresponding hazard ratios were estimated using a Cox-proportional hazard regression model without additional covariates. The multiple test Hochberg procedure[17] was applied to control the family-wise error rate across the three comparisons of asunercept plus SOC versus SOC alone. Time-to-event data were visualised by Kaplan–Meier plots.

A sensitivity analysis was performed for the primary endpoint, changing the definition to time to clinical improvement of at least one category on the WHO scale from randomisation on two consecutive days or without confirmation of improvement on the second consecutive day if the patient was discharged from the hospital on the day of improvement.

Several *post hoc* subgroup analyses have been conducted, including analyses of the primary and secondary endpoints by subgroups of patients according to their WHO scale at inclusion.

The significance level was defined as 2.5% (one-sided). Secondary endpoint analyses were considered exploratory. p-values and 95% confidence intervals (CIs) were used for descriptive purposes only. Missing data or dropouts were not replaced and, therefore, during the statistical analysis the missing data were not considered. Study data were analysed using SAS software, version 9.4.

The ASUNCTIS study is registered on EudraCT (2020-001887-27) and ClinicalTrials.gov (NCT04535674). It was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice and adhered to the CONSORT reporting guidelines.

Role of the funding source

The funder co-coordinated the study design, protocol writing, and recruitment of study centres; conducted and supervised the study, and the data collection, analysis, and interpretation; conducted and supervised the writing of the reports; and co-supervised the writing of this manuscript as well as submission of the manuscript for publication.

Results

Between October 9, 2020, and September 24, 2021, 440 patients were screened for study eligibility. Of the 438 patients who were randomised (185 in Spain and 253 in Russia; 9–90 patients per site), 110 were allocated to SOC alone, 109 to SOC plus asunercept 25 mg, 109 to SOC plus asunercept 100 mg, and 110 to SOC plus asunercept 400 mg (hereafter referred to as the asunercept 25 mg, asunercept 100 mg, and asunercept 400 mg arms, respectively). In total, 350 patients completed the study or fully recovered before the end of therapy (last patient completed the study on December 21, 2021), and 88 terminated the study early (Fig. 1). Reasons for termination were death (n = 34), investigator decision (n = 24), loss to follow-up (n = 11), AE/SAE (n = 9), patient decision (n = 7), and other (n = 3). As at the time of the study no vaccines were widely available, only one patient (in the 400 mg asunercept group) received vaccination.

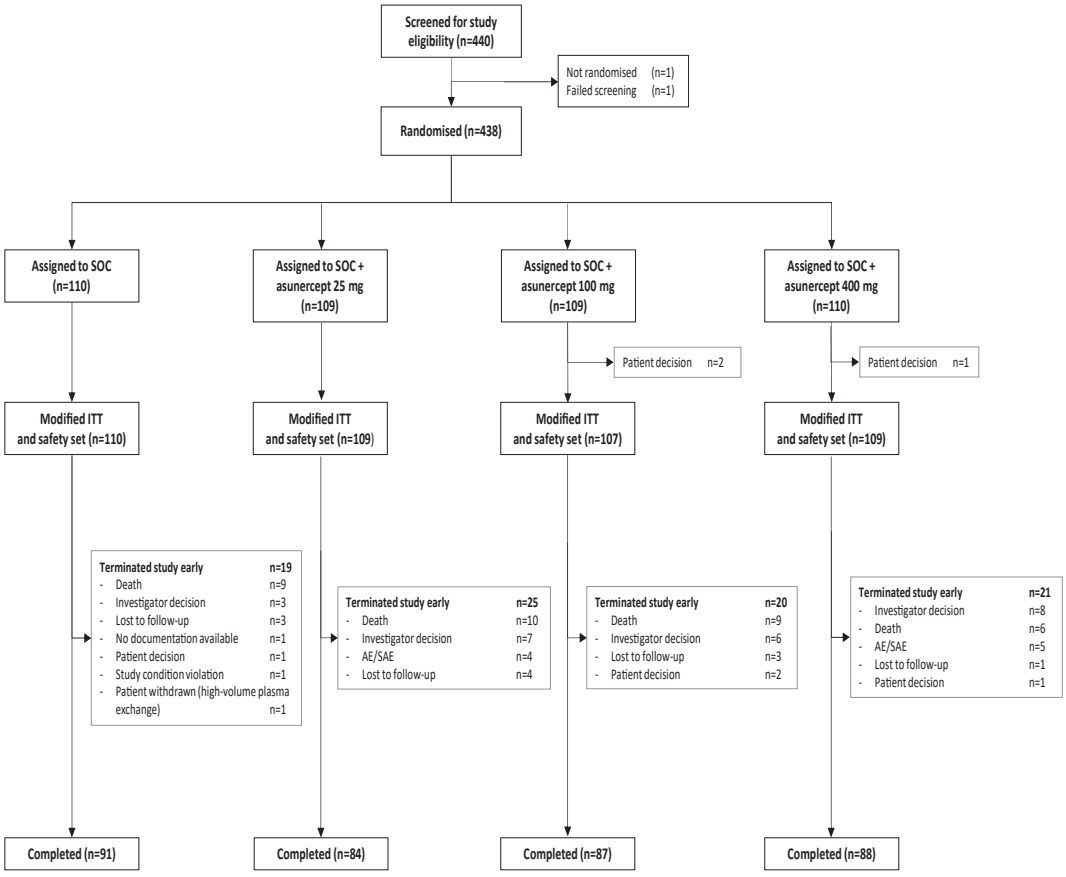

***Fig. 1:* Flow of patients through the study**. AE, adverse event. ITT, intent-to-treat. mITT, modified intent-to-treat. PP, per protocol. SAE, serious adverse event. SOC, standard of care.

The mITT set included 435 patients (three patients were excluded due to premature study discontinuation before the start of therapy) and the PP set included 431 patients (four patients were excluded due to major deviations at randomisation caused by incorrect WHO classification). The safety set included the same 435 patients as the mITT set. Baseline characteristics are summarised in Table 1. The baseline characteristics were slightly imbalanced regarding known prognostic factors (e.g., age, hypertension, cardiac disorders, and co-treatments). An unspecified analysis of comorbidities at screening using the Charlson Comorbidity Index (CCI) showed a higher CCI in patients receiving 100 mg and especially 400 mg asunercept (mean 2.10 ± standard deviation 1.89 and 2.41 ± 1.94 CCI points per patient, respectively) versus the SOC group (1.97 ± 1.67, p = 0.59 vs 100 mg and p = 0.073 vs. 400 mg group, t-test).[18]

Regarding the primary endpoint, a sustained clinical improvement of at least one WHO category was achieved in 257 patients (59.1%) in the mITT set. The percentage of patients who met the primary endpoint was higher in the asunercept arms (61–63%) than in the SOC arm (51%) in the mITT set (Table 2). The median time to clinical improvement estimated by the Kaplan–Meier method was shorter in the asunercept arms (25 mg arm: 9 [95% CI 6–12] days; one-sided p-value: 0.041; 100 mg arm: 8 [7–12] days; one-sided p-value: 0.028; and 400 mg arm: 8 [7–11] days; one-sided p-value: 0.036) than in the SOC arm (13 [9–20] days) for the mITT set (Table 2; Fig. 2). The Cox-proportional hazard model and comparison of asunercept 25 mg, 100 mg, and 400 mg arms versus SOC using the log-rank test demonstrated an adjusted one-sided p-value of 0.041 in the mITT set.

An additional *post hoc* comparison of Kaplan–Meier curves between the control/SOC group and the combined asunercept group confirmed these results, with the median time to improvement in the combined

	SOC (N = 110)	SOC + asunercept 25 mg (N = 109)	SOC + asunercept 100 mg (N = 107)	SOC + asunercept 400 mg (N = 109)
Country				
Spain	47 (42.7%)	44 (40.4%)	51 (47.7%)	42 (38.5%)
Russia	63 (57.3%)	65 (59.6%)	56 (52.3%)	67 (61.5%)
Race				
White/Caucasian	108 (98.2%)	108 (99.1%)	107 (100%)	103 (94.5%)
Black	1 (0.9%)	0	0	2 (1.8%)
Asian/Oriental	0	0	0	1 (0.9%)
Other	1 (0.9%)	1 (0.9%)	0	3 (2.8%)
Ethnicity				
Hispanic or Latino	15 (13.6%)	14 (12.8%)	12 (11.2%)	8 (7.3%)
Not Hispanic or Latino	95 (86.4%)	95 (87.2%)	95 (88.8%)	101 (92.7%)
Sex				
Male	61 (55.5%)	61 (56.0%)	63 (58.9%)	60 (55.0%)
Female	49 (44.5%)	48 (44.0%)	44 (41.1%)	49 (45.0%)
Age (years)				
Mean (SD)	57.2 (13.1)	56.2 (13.3)	55.9 (13.5)	59.1 (12.7)
Median	58.8	58.0	57.0	62.0
Min/Max	22/83	25/82	22/82	34/94
Age ≥65 years	35 (31.8%)	36 (33%)	33 (30.8%)	44 (40.4%)
BMI (kg/m^2)				
Mean (SD)	29.8 (4.7)	29.9 (5.4)	29.9 (5.7)	30.6 (4.8)
Median	29.2	28.7	29.3	30.5
Min/Max	20.1/46.7	18.4/48.0	20.7/46.7	18.9/46.6
Comorbidities				
Hypertension	34 (30.9%)	29 (26.6%)	39 (36.4%)	45 (41.3%)
Diabetes	12 (10.9%)	5 (4.6%)	8 (7.5%)	9 (8.3%)
Obesity	16 (14.5%)	12 (11.0%)	21 (19.6%)	19 (17.4%)
Cardiac disorders	18 (6.4%)	26 (23.9%)	28 (26.2%)	30 (27.5%)
Chronic obstructive lung disease	1 (0.9%)	3 (2.8%)	2 (1.9%)	1 (0.9%)
Randomisation characteristics				
Duration of hospitalisation before randomisation (days), Mean (SD)	2.8 (2.2)	2.9 (2.2)	3.0 (2.5)	3.1 (2.1)
ICU patients at randomisation	9 (8.2%)	13 (11.9%)	11 (10.3%)	9 (8.3%)
Transfer to ICU within 3 days of randomisation	5 (4.5%)	6 (5.5%)	6 (5.6%)	10 (9.2%)
Patients randomised >3 days after hospitalisation	15 (13.6%)	21 (19.3%)	22 (20.6%)	24 (22.0%)
9-point WHO COVID-19 ordinal scale				
Severity at baseline WHO 4	87 (79.1%)	87 (79.8%)	83 (77.6%)	85 (78.0%)
Severity at baseline WHO 5	22 (20.0%)	21 (19.3%)	22 (20.6%)	23 (21.1%)
Severity at baseline WHO 6	1 (0.9%)	1 (0.9%)	2 (1.9%)	1 (0.9%)
Co-treatments				
Antivirals (including remdesivir)	33 (30.0%)	32 (29.4%)	35 (32.7%)	36 (33.0%)
Corticosteroids	93 (84.5%)	81 (74.3%)	85 (79.4%)	87 (79.8%)
Tocilizumab	9 (8.2%)	7 (6.4%)	10 (9.3%)	4 (3.7%)

BMI, body mass index. COVID-19, coronavirus disease 2019. ICU, intensive care unit. Max, maximum. Min, minimum. mITT, modified intent-to-treat. SD, standard deviation. SOC, standard of care. WHO, World Health Organization.

Table 1: **Patient baseline characteristics and demographics (modified intent-to-treat set).**

asunercept group being shorter than in the SOC group (8 [95% CI 7–10] vs 13 [9–20] days, respectively). Applying the same statistical methods as before, there was significantly faster clinical improvement in the combined asunercept group (i.e., asunercept at all doses; one-sided p-value 0.019 in the mITT population; Fig. 3, Table 3).

The majority of the included patients had WHO category 4 at inclusion and we analysed clinical outcomes in this subgroup. First, in this group (mITT set) the median time to clinical improvement, as analysed by the Kaplan–Meier function, was 30–40% shorter in the asunercept arms (8–9 days) compared with the SOC arm (14 days). Second, clinical improvement was seen in

mITT	SOC (N = 110)	SOC + asunercept 25 mg (N = 109)	SOC + asunercept 100 mg (N = 107)	SOC + asunercept 400 mg (N = 109)
Number of clinical improvements, n (%)	56 (50.9%)	67 (61.5%)	65 (60.7%)	69 (63.3%)
Median (95% CI) time to clinical improvement[a] (days)	13 (9–20)	9 (6–12)	8 (7–12)	8 (7–11)
HR estimate (95% CI)	1 (–)	1.36 (0.96–1.94)	1.39 (0.97–1.98)	1.36 (0.96–1.94)
p-value (one-sided) of log-rank test	–	0.041	0.028	0.036
Adjusted p-value of log-rank test (using the Hochberg procedure)	–	0.041	0.041	0.041

CI, confidence interval. HR, hazard ratio. mITT, modified intent-to-treat. SOC, standard of care. –, not applicable.
[a]Kaplan–Meier estimate.

Table 2: **Summary of the Cox-proportional hazard model and comparison between the asunercept and SOC arms using a log-rank test for the time to clinical improvement.**

more patients in each individual asunercept treatment arm (60.2–61.2%) compared with the SOC arm (47.1%). The asunercept 100 mg arm versus SOC showed a hazard ratio of 1.53 (95% CI 1.01–2.31; one-sided p-value = 0.016) for time to clinical improvement (Table 4).

The sensitivity analysis (i.e., time to clinical improvement of at least one category on the WHO scale from randomisation, including patients discharged from hospital for whom improvement was observed for only 1 day before hospital discharge) confirmed the primary and subgroup analyses, indicating a faster clinical improvement in the asunercept arms compared with the SOC arm. Median time to clinical improvement according to Kaplan–Meier function was 9 days in the SOC arm and 7 days in each of the individual asunercept arms. The number of patients with clinical improvement were homogeneous in the asunercept treatment arms and were higher than in the SOC group (Table 5). In summary, the sensitivity analysis confirmed the tendency towards faster clinical improvement in the asunercept treatment arms as compared with the SOC group.

With respect to secondary endpoints, all-cause mortality was not significantly lower in the asunercept groups compared with the SOC group (Table 6). A post-hoc multivariate analysis did not find any meaningful differences when comparing the study arms. Other secondary endpoints regarding oxygenation, ventilation, hospitalisation, and ICU admission did not differ between treatment groups (Supplementary Tables S5–S11). With regard to the NEWS score, there was a tendency toward faster clinical recovery (Mean of 7.1, 5.6, 6.4 and 6.5 days in the SOC, 25 mg, 100 mg and 400 mg treatment groups, respectively, until time of hospital discharge or to a NEWS ≤2 maintained for 2 days) which was, however, not significant.

With regard to the effect of asunercept on total lymphocyte counts, at study onset, 52.6% of patients had lymphocytopenia (lymphocyte counts $<1 \times 10^9$/L) with a median lymphocyte count of 0.95×10^9/L, standard deviation (SD) = 0.81×10^9/L. When stratified by the different asunercept treatment arms (Fig. 4a), there appeared to be a dose-dependent effect on lymphocyte recovery in the 100 mg and 400 mg asunercept groups. To further investigate this effect, a pharmacokinetic/pharmacodynamic modelling analysis was performed.

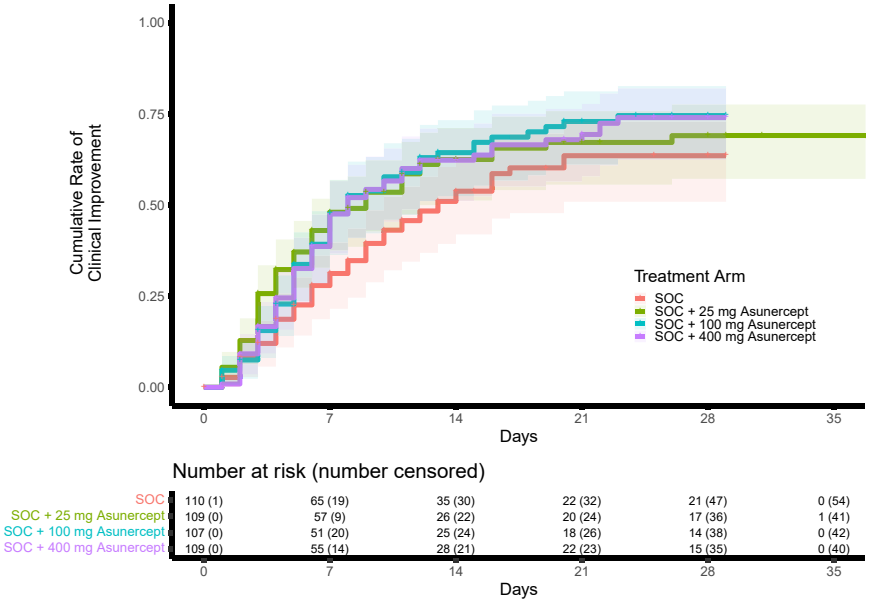

Fig. 2: **Kaplan–Meier cumulative rate and its 95% confidence interval for the time to clinical improvement.** SOC, standard of care.

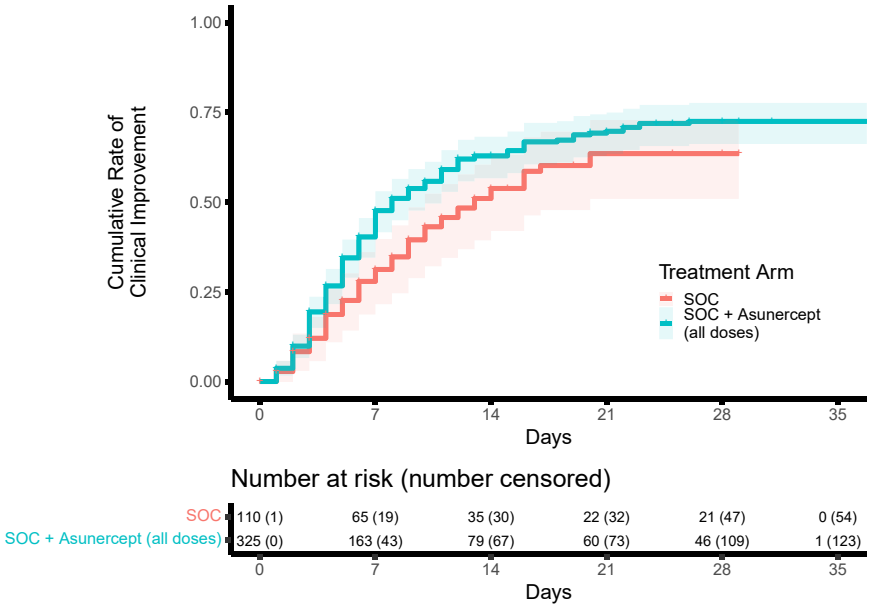

Fig. 3: **Kaplan–Meier cumulative rate and its 95% confidence interval for the time to clinical improvement (asunercept all doses versus SOC, mITT set).** mITT, modified intent-to-treat. SOC, standard of care.

The Supplementary Results (and Supplementary Table S12 and Figure S2) show the development of this model to determine the correlation between asunercept treatment and lymphocytes counts. Simulations of plasma concentrations and lymphocyte counts for all asunercept treatment arms also suggested a dose-dependent effect (Fig. 4b and c). In this model, asunercept had a significant ($p < 0.0001$) impact on the regeneration of the lymphocyte count, following a saturable response relationship with a half-maximum effective concentration (EC_{50}) value of 2.57 mg/L. Lymphocyte regeneration was characterised by a 1.9-day lag between asunercept exposure and an increase in circulating lymphocytes. Examples of single patient analyses including simulation are given in Supplementary Figure S3.

Safety analyses comprised the incidence of adverse events (AE) and serious adverse events (SAE), discontinuation or temporary suspension of therapy, changes in white cell count, hemoglobin, platelets, creatinine, glucose, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and uric acid over time. In total, 290 AEs were registered in 145 patients who received at least one dose of the study treatment: 77 AEs in 37 (33.6%) patients in the SOC group, 80 AEs in 38 (34.9%) patients in the SOC plus 25 mg asunercept group, 61 AEs in 35 (32.7%) patients in the SOC plus 100 mg asunercept group and 72 AEs in 35 (32.1%) patients in the SOC plus 400 mg asunercept group. Of the 290 AEs, 79 AEs were qualified as SAEs and were registered in 61 patients: 23 SAEs in 17 (15.5%) patients in the SOC group, 17 SAEs in 15 (13.8%) patients in the SOC plus 25 mg asunercept group, 16 SAEs in 15 (14.0%) patients in the SOC plus 100 mg asunercept group and 23 SAEs in 14 (12.8%) patients in the SOC plus 400 mg asunercept group. AEs and SAEs occurred in all groups with a similar rate. There were no clinically relevant negative dynamics in laboratory parameters (including white cell count, hemoglobin, platelets, creatinine, glucose, total bilirubin, ALT, AST, uric acid). Based on the AE analysis, a similar safety profile was observed in all treatment arms. Only two SAEs seen in one patient (klebsiella infection and acute kidney failure) were qualified by the investigator as possibly related to asunercept. These events led to the discontinuation of the drug administration to this patient on the discretion of the investigator. All other SAEs were assessed as asunercept-unrelated. In summary, no trend or pattern

mITT	SOC (N = 110)	Asunercept, all doses (N = 325)
Number of clinical improvements, n (%)	56 (50.9%)	201 (61.8%)
Median (95% CI) time to clinical improvement[a] (days)	13 (9–20)	8 (7–10)
HR estimate (95% CI)	1 (–)	1.369 (1.018–1.842)
p-value (one-sided) of log-rank test	–	p = 0.019

CI, confidence interval. HR, hazard ratio. mITT, modified intent-to-treat. SOC, standard of care. –, not applicable.
[a]Kaplan–Meier estimate.

Table 3: **Summary of the Cox-proportional hazard model and comparison of Kaplan–Meier curves between treatment and control groups using a log-rank test for the time to clinical improvement (asunercept all doses versus SOC, mITT set).**

mITT set	SOC (N = 110)	Asunercept 25 mg (N = 109)	Asunercept 100 mg (N = 107)	Asunercept 400 mg (N = 109)	Total (% of mITT)
Clinical Status according to WHO ordinal scale at randomisation: oxygen by mask or nasal prongs (Score 4)					
n (%)	87 (79.1%)	87 (79.8%)	83 (77.6%)	85 (78.0%)	342 (78.6%)
Number of clinical improvements, n (%)	41 (47.1%)	53 (60.9%)	50 (60.2%)	52 (61.2%)	
Median (95% CI) time to clinical improvement[a] (days)	14 (9–NE)	9 (6–13)	8 (6–11)	8 (7–12)	
HR estimate (95% CI)	1 (–)	1.44 (0.958–2.165)	1.531 (1.012–2.314)	1.411 (0.937–2.125)	
p-value (one-sided) of log-rank test	–	p = 0.037	p = 0.016	p = 0.044	
Adjusted p-value of log-rank test (using the Hochberg procedure)		p = 0.044	p = 0.044	p = 0.044	

CI, confidence interval. HR, hazard ratio. mITT, modified intent-to-treat. SOC, standard of care. NE, not estimable. WHO, World Health Organization. –, not applicable. [a]Kaplan–Meier estimate.

Table 4: **Summary of the Cox-proportional hazard model for the time to clinical improvement in the WHO 4 subgroup (mITT set) (342/435 patients; 78.6%).**

was identified among serious and non-serious AEs for all treatment arms. Asunercept was well tolerated and shows a favourable safety profile. In addition, Asunercept has previously been demonstrated to be well tolerated in phase 1 and 2 trials in healthy individuals and patients with glioblastoma or myelodysplastic syndromes.[19–21]

Discussion

In the phase II ASUNCTIS study, we investigated the efficacy, safety and tolerability of asunercept, a CD95L-blocking agent in hospitalised patients with COVID-19. Of note, this was also the first ever clinical trial to investigate the effects of blocking this pathway in an infectious disease. Importantly, this study was conducted during the phase of the pandemic when highly pathogenic variants of SARS-CoV-2 were prevalent. Asunercept treatment caused only few treatment-related adverse events with mostly mild or moderate severity and demonstrated a good safety profile. While statistical significance on the primary endpoint was not reached when comparing the SOC arm with the individual asunercept plus SOC arms, a trend for faster clinical improvement was observed especially in the 100 mg asunercept arm, with a reduction in the median time to sustained clinical improvement of 4–5 days compared with SOC alone. In a post-hoc analysis the combined asunercept dose arms showed a statistically significantly faster clinical improvement in the treatment group compared with SOC. However, it has to be noted that this result is not protected against the pre-specified type-1 error as the analysis was done as a post-hoc analysis and the primary endpoint of the study was missed. COVID-19 might be specifically suitable as a target disease, because significantly elevated levels of CD95L were found in bronchoalveolar fluids of these patients.[14] Of note, elevated CD95L concentrations were also found in patients with lung failure caused by influenza A virus, suggesting a potential role of CD95L also for disease caused by this virus.

Importantly, patients treated with asunercept neither had an increased rate of super-infections nor an inability to control the infection with SARS-CoV-2 implying that,

mITT set	SOC (N = 110)	Asunercept 25 mg (N = 109)	Asunercept 100 mg (N = 107)	Asunercept 400 mg (N = 109)
Number of clinical improvements, n (%)	81 (73.6%)	85 (78.0%)	83 (77.6%)	87 (79.8%)
Median (95% CI) time to clinical improvement[a] (days)	9 (7–11)	7 (5–9)	7 (5–8)	7 (6–8)
HR estimate (95% CI)	1 (–)	1.201 (0.886–1.629)	1.235 (0.909–1.677)	1.191 (0.88–1.613)
p-value (one-sided) of log-rank test	–	p = 0.107	p = 0.074	p = 0.113
Adjusted p-value of log-rank test (using the Hochberg procedure)	–	p = 0.113	p = 0.113	p = 0.113

CI, confidence interval. HR, hazard ratio. mITT, modified intent-to-treat. SOC, standard of care. –, not applicable. [a]Kaplan–Meier estimate.

Table 5: **Summary of the Cox-proportional hazard model and comparison of Kaplan–Meier curves between treatment and control groups using a log-rank test for the time to clinical improvement (mITT set).**

at least in the context of COVID-19, blocking CD95L does not compromise host immune protection. This is in line with observations in mice, in which inhibition of the CD95/CD95L system did not interfere with the clearance of pulmonary bacterial infections.[22] It should be noted that other immunomodulatory treatments, such as tumour necrosis factor blockers, were previously shown to enhance the risk of serious infections by diverse pathogens, including fungi, bacteria and viruses, in treated patients.[23,24]

The CD95/CD95L system is known to mediate activation-induced cell death (AICD) of lymphocytes.[7] Thus, asunercept might lead to a reduction of CD95L-mediated lymphocyte death, thereby limiting the otherwise pronounced lymphocytopenia that is observed in the majority of patients with moderate-to-severe COVID-19.[25–27] This might be of interest, as the degree of lymphocytopenia is positively correlated with disease progression and mortality[9] and a strong positive correlation has been observed between levels of CD95 expression on T cells and T-cell apoptosis in patients with COVID-19.[28] To approach this question within the cohort of our study we performed a post-hoc *in silico* analysis of the interaction between lymphocyte count and asunercept plasma levels. In this model, asunercept had a dose-dependent effect ($p < 0.001$) on the restoration of reduced lymphocyte counts. Our semi-mechanistic model was developed based on the widely used approach for modelling blood cell development kinetics originating from stem cells. This model was, however, developed in healthy volunteers, which is an obvious limitation regarding the present study's severely ill patient population.[29–31] Nevertheless and although one should not overestimate such model-derived results, they may be interpreted as supportive of the hypothesis that asunercept may diminish lymphocytopenia in patients with COVID-19, possibly by interfering with CD95L-mediated T-cell death.

mITT set	SOC (N = 110)	Asunercept 25 mg (N = 109)	Asunercept 100 mg (N = 107)	Asunercept 400 mg (N = 109)
15-day mortality, n (%)	9 (8.2)	7 (6.4)	5 (4.7)	5 (4.6)
RD[a] (95% CI)	–	−1.8 (−8.6, 5.1)	−3.5 (−10.0, 3.0)	−3.6 (−10.0, 2.9)
29-day mortality, n (%)	13 (11.8)	11 (10.1)	10 (9.3)	10 (9.2)
RD[a] (95% CI)	–	−1.7 (−10.0, 6.5)	−2.5 (−10.6, 5.7)	−2.6 (−10.8, 5.5)
60-day mortality, n (%)	13 (11.8)	13 (11.9)	10 (9.3)	10 (9.2)
RD[a] (95% CI)	–	0.1 (−8.5, 8.7)	−2.5 (−10.6, 5.7)	−2.6 (−10.8, 5.5)
90-day mortality, n (%)	13 (11.8)	13 (11.9)	10 (9.3)	10 (9.2)
RD[a] (95% CI)	–	0.1 (−8.5, 8.7)	−2.5 (−10.6, 5.7)	−2.6 (−10.8, 5.5)

[a]RD, Risk difference vs SOC.

Table 6: **All-Cause Mortality (mITT set).**

The strengths of our study are that it included a large number of patients (n = 435 in the mITT) representative of the patient population most severely affected by the virus, use of a randomised design with an active control arm comprising SOC including corticosteroids and remdesivir, and measured efficacy using clinically meaningful endpoints. However, some limitations should be considered. First, in this open-label study there was some heterogeneity in the recruited patient population with regard to the total dose of asunercept received (as the treatment ended at hospital discharge) and the time to randomisation. Second, at the time of study conduct there was no clearly defined SOC treatment. Third, there were imbalances in certain baseline characteristics regarding prognostic factors. A multivariate analysis was undertaken including those prognostic factors showing imbalances at baseline into a Cox proportional hazards regression model. The results of this analysis did not lead to meaningful differences

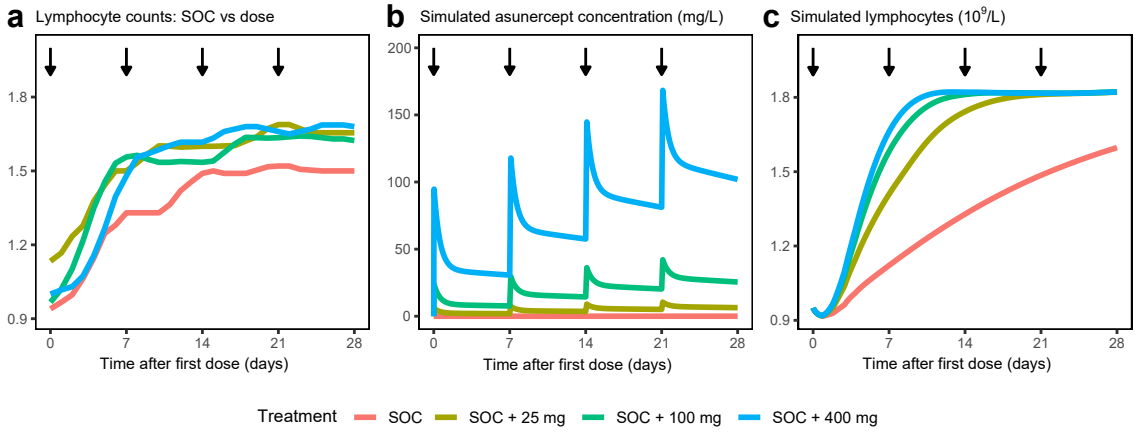

***Fig. 4:* Correlation of lymphocyte counts and asunercept administration. a: 3-day rolling average of the median observed lymphocyte counts stratified by treatment arm. b: Simulation results for plasma asunercept concentrations. c: Simulation results for lymphocyte counts in correlation to asunercept concentrations**. Arrows indicate asunercept dosing. (Colour: red = SOC, olive = SOC + 25 mg, green = SOC + 100 mg, blue = SOC + 400 mg).

compared to the primary analysis (data not shown). Fourth, at inclusion the majority of patients were of WHO grade 4 (78.6%). Conclusions about the effects of asunercept in more severe cases (WHO grade 5 or 6) are limited by the small sample size. Fifth, due to incomplete data sets we utilised a mathematical modelling for the statistical analysis of lymphocyte counts. This analysis is limited by the lack of direct pharmacokinetic (PK) data from COVID-19 patients. The ASUNCTIS study did not allow for the collection of the necessary plasma concentrations to compare PK across healthy individuals and COVID-19 patients. Therefore, the application of the existing PK model to COVID-19 patients is an extrapolation. Nevertheless, the model includes various covariates designed to reflect the physiological variations among individuals, which helps in approximating individual plasma concentrations in the absence of specific COVID-19 patient data. Sixth, as the study was done in a pandemic caused by a then recently emerged novel virus, using an innovative, non-licensed drug for the first time in this or similar indications, choice of primary endpoints and calculation of the study size was based on limited data. Therefore, exploratory post-hoc analyses were conducted to determine if their results supported the trends observed in the primary data.

In summary, this open-label phase 2 study was designed to assess safety, tolerability and preliminary efficacy of the compound; to find the optimal dose of the compound to be administered to patients with moderate to severe COVID-19; to identify the patient cohort that responds best to the study drug and, finally, to collect data on the presumed mode of action of the compound. These goals have been achieved. Whilst the study missed to reach the primary endpoint with statistical significance, promising trends and an excellent safety profile were, however, observed. This warrants future clinical development of this compound in viral infections characterised by lymphocytopenia and acute respiratory distress syndromes associated with high CD95L levels in the bronchoalveolar lavages of patients.

Contributors

TH, HW, and C Schoergenhofer accessed and verified the data. JP, DdM, MB, TH, and HW conceived the scientific rationale for the study. JP, DdM, JRP, MB, MPRS, MK, AK, C Straub, C Schoergenhofer, BJ, TH, and HW carried out the original design of the study, including drafting the study protocol and writing of ethical and legal aspects. TH, BJ, C Schoergenhofer, JP, MB, and HW formed part of the ASUNCTIS steering committee, chaired by HW. The final study design and protocol was elaborated by MPRS, JRP, C Schoergenhofer, BJ, FH, JP, DdM, MB, AK, C Straub, MK, HW, and TH, coordinated by HW, MB, and TH. MPRS, JRP, JP, DdM, and HW contributed to the recruitment of hospitals involved in the study in Spain. MPRS and JRP enrolled patients, and MPRS served as Principal Investigator of the study. MK served as a statistical consultant during the study and reviewed the statistical results. CD and TL analysed the influence of asunercept on lymphocyte counts. HF, MT, JRP, C Schoergenhofer, JP, DdM, BJ, MB, AK, C Straub, MK, HW and TH contributed to the writing of the manuscript, which TH coordinated. All authors had direct and full access to all the data in the study, verified the full dataset, accept responsibility to submit for publication, and read and approved the manuscript. All authors confirm that they had direct and full access to the statistical analysis report and accept responsibility for the accuracy of the underlying data reported in this manuscript.

Data sharing statement

The study protocol can be found in the supplementary data. De-identified individual participant data relating to the main study endpoints will be made available for fellow researchers upon reasonable request to the study sponsor or the corresponding author. Data will be available from 3 months after manuscript publication for a period of 5 years. All individuals who request data access are required to provide a methodologically sound justification.

Declaration of interests

C Schoergenhofer and FH received honoraria from the sponsor for participation in the DSMB. CD is an employee of Saarmetrics GmbH which received payments from Apogenix GmbH for the analysis of the influence of asunercept on lymphocyte counts described in the manuscript. TL is a stakeholder of Saarmetrics GmbH which received payments from Apogenix GmbH for the analysis of the influence of asunercept on lymphocyte counts described in the manuscript. Saarmetrics received consulting fees for CD's and TL's work as consultants for Apogenix GmbH. AK and C Straub were employed by Apogenix GmbH at the time the study was conducted. JRPP, C Schoergenhofer, MB and BJ received funding for clinical trials from the sponsor. MK is an employee of Cytel Inc. and worked as a statistical consultant for Apogenix GmbH. HF, HW and TH are co-founders and shareholders of Apogenix GmbH. HW also worked as a consultant for Apogenix GmbH. JP, DdM and MT declare that they have no conflicts of interest.

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