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ORIGINAL RESEARCH ARTICLE

Effect of the P-Selectin Inhibitor Crizanlizumab on Survival Free of Organ Support in Patients Hospitalized for COVID-19: A Randomized Controlled Trial

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BACKGROUND: COVID-19 has been associated with endothelial injury, resultant microvascular inflammation and thrombosis. Activated endothelial cells release and express P-selectin and von Willebrand factor, both of which are elevated in severe COVID-19 and may be implicated in the disease pathophysiology. We hypothesized that crizanlizumab, a humanized monoclonal antibody to P-selectin, would reduce morbidity and death in patients hospitalized for COVID-19.

METHODS: An international, adaptive, randomized controlled platform trial, funded by the National Heart, Lung, and Blood Institute, randomly assigned 422 patients hospitalized with COVID-19 with moderate or severe illness to receive either a single infusion of the P-selectin inhibitor crizanlizumab (at a dose of 5 mg/kg) plus standard of care or standard of care alone in an open-label 1:1 ratio. The primary outcome was organ support–free days, evaluated on an ordinal scale consisting of the number of days alive free of organ support through the first 21 days after trial entry.

RESULTS: The study was stopped for futility by the data safety monitoring committee. Among 421 randomized patients with known 21-day outcomes, 163 patients (77%) randomized to the crizanlizumab plus standard-of-care arm did not require any respiratory or cardiovascular organ support compared with 169 (80%) in the standard-of-care–alone arm. The adjusted odds ratio for the effect of crizanlizumab on organ support–free days was 0.70 (95% CI, 0.43–1.16), where an odds ratio >1 indicates treatment benefit, yielding a posterior probability of futility (odds ratio <1.2) of 98% and a posterior probability of inferiority (odds ratio <1.0) of 91%. Overall, there were 37 deaths (17.5%) in the crizanlizumab arm and 27 deaths (12.8%) in the standard-of-care arm (hazard ratio, 1.33 [95% CrI, 0.85-2.21]; [probability of hazard ratio>1] = 0.879).

CONCLUSIONS: Crizanlizumab, a P-selectin inhibitor, did not result in improvement in organ support–free days in patients hospitalized with COVID-19.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT04505774.

Key Words: COVID-19 ■ crizanlizumab ■ P-selectin

Editorial, see p 391

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ORIGINAL RESEARCH ARTICLE

Clinical Perspective

What Is New?

- Endothelial injury and activation are thought to play a role in the pathophysiology of severe COVID-19 and may mediate microvascular inflammation and thrombosis. We randomized hospitalized patients with COVID-19 to receive the P-selectin inhibitor crizanlizumab or usual care, with a primary end point of organ support–free days.
- Crizanlizumab did not lead to a reduction in the number of organ support–free days or improvement in any secondary end points. The study was stopped for futility by the data safety monitoring board.

What Are the Clinical Implications?

- Crizanlizumab did not result in improvement in organ support–free days or in-hospital death for patients hospitalized with moderate or severe COVID-19.
- These data do not support use for reducing complications in patients hospitalized for COVID-19, and suggest that inhibition of P-selectin is unlikely to benefit patients with moderate to severe COVID-19.

Nonstandard Abbreviations and Acronyms

ACTIV-4a	Accelerating COVID-19 Therapeutic Interventions and Vaccines 4 ACUTE
BNP	B-type natriuretic peptide
CrI	credible interval
NT-proBNP	N-terminal pro-B-type natriuretic peptide
OR	odds ratio
Pr	probability

COVID-19 is associated with cardiovascular complications such as myocardial infarction, stroke, venous thromboembolism, and microvascular thrombosis. Endothelial injury leading to microvascular inflammation and thrombosis has been implicated in the pathophysiology of COVID-19, including the severe respiratory complications associated with the disease.[1,2] Activated endothelial cells release P-selectin and von Willebrand factor, both of which are elevated in severe COVID-19.[3,4] When expressed on the endothelial surface, P-selectin promotes inflammation and thrombosis, mediates leukocyte adherence, and anchors ultralong von Willebrand factor multimers, facilitating platelet adherence to the vessel wall. Similarly, P-selectin expressed by platelets mediates platelet interactions with endothelial cells and leukocytes. In addition, P-selectin may play a role in neutrophil extracellular trap formation,[5] which has also been linked to microvascular and macrovascular thrombosis.[6] These mechanisms have implicated P-selectin as a key potential mediator in the microvascular inflammation and thrombosis seen in more severe forms of COVID-19.

Crizanlizumab is a humanized monoclonal antibody to P-selectin that has been approved by the US Food and Drug Administration for the prevention of vaso-occlusive crisis in sickle cell disease.[7] In a pilot trial on patients hospitalized for COVID-19, treatment with crizanlizumab appeared safe, reduced soluble P-selectin levels, and was associated with several biomarker changes suggestive of increased fibrinolysis and decreased thrombin formation.[8] Therefore, we tested the hypothesis that crizanlizumab would reduce morbidity and death in patients hospitalized for COVID-19 in an international, adaptive, randomized controlled platform trial.

METHODS

Trial Design and Oversight

The ACTIV-4a trial (Accelerating COVID-19 Therapeutic Interventions and Vaccines 4 ACUTE) is an international, multicenter, open-label, Bayesian, adaptive randomized platform trial funded by the National Heart, Lung, and Blood Institute. The trial conduct is overseen by a joint clinical coordinating center at the New York University Grossman School of Medicine (study chairs office), the University of Pittsburgh School of Medicine (study co-chairs office), Brigham and Women's Hospital, and Mid-America Heart Institute, as well as a data coordinating center at the University of Pittsburgh School of Medicine. Patients were enrolled across several clinical trial networks. The study drug was provided by Novartis, which had no role in the design and execution of the trial or analysis of the data. This platform trial tested several therapeutic domains (sequentially and concurrently) added to standard of care, including therapeutic-dose heparin,[9] $P2Y_{12}$ inhibition,[10] sodium-glucose cotransporter-2 inhibition, and P-selectin inhibition with crizanlizumab. An independent data and safety monitoring board oversaw the trial conduct and patient safety. Local or central institutional review board approval was provided at each site. Details about the trial design are available in the protocol and the statistical analysis plan (Supplemental Appendix). The trial is registered on ClinicalTrials.gov (NCT04505774).

Study Population

Eligibility requirements at screening included an age of at least 18 years and hospitalization for confirmed SARS-CoV-2 infection with an expected hospital stay of >72 hours. Patients were required to be enrolled within 72 hours of hospital admittance or within 72 hours of a positive COVID-19 test. At the time of randomization, enrolled patients were prospectively stratified into either a severe illness cohort, requiring intensive care–level support, or a moderate illness cohort, requiring hospitalization but not intensive care–level support. Intensive care–level support was defined as the use of respiratory or cardiovascular organ support, including oxygen through a high-flow nasal cannula at ≥20 L/min, noninvasive or invasive mechanical

ventilation, vasopressors, inotropes, or extracorporeal membrane oxygenation. To be eligible for the moderate illness cohort, patients were additionally required to be either ≥65 years of age or to meet at least 2 of the following prespecified enrichment criteria: oxygen supplementation of >2 L/min; body mass index ≥35 kg/m^2; estimated glomerular function rate ≤60 mL·min^{-1}·1.73 m^{-2} body surface area; elevated D-dimer level ≥2-fold the upper limit of normal of the site; elevated cardiac troponin level ≥2-fold the upper limit of normal of the site; elevated natriuretic peptides (BNP [B-type natriuretic peptide] ≥100 pg/mL or NT-proBNP [N-terminal pro-B-type natriuretic peptide] ≥300 pg/mL); elevated C-reactive protein level ≥50 mg/L; history of type 2 diabetes; or history of heart failure (regardless of ejection fraction).

Exclusion criteria included imminent death, requirement for chronic mechanical ventilation through tracheostomy before hospitalization, pregnancy, current or planned breastfeeding, conditions precluding the use of crizanlizumab such as uncontrolled bleeding or severe anemia (hemoglobin <4 g/dL), and open-label treatment with crizanlizumab within the past 3 months. Detailed inclusion and exclusion criteria are provided in the protocol (Supplemental Appendix).

Procedures

All patients or patients' legal representatives provided written informed consent. Patients who met the inclusion and exclusion criteria were randomly assigned to a single infusion of the P-selectin inhibitor crizanlizumab (at a dose of 5 mg/kg) plus standard of care or standard of care alone in an open-label 1:1 ratio. Randomization was concealed through an interactive voice- or Web-response system (Worldwide Clinical Trials) and stratified by hospital site and disease severity using eSOCDAT software (Socar Research). A minimization procedure was instituted after enrollment of the first 20 patients (who were allocated randomly) to equilibrate the imbalance across the active enrollment domains accounting for the severity group of a new patient. Those randomized to crizanlizumab received a single infusion after randomization of 5 mg/kg in 100 mL of 0.9% sodium chloride or 5% dextrose over a period of 30 minutes. Standard care recommendations included therapeutic-dose heparin for patients in the moderate illness cohort and prophylactic-dose heparin for those in the severe illness cohort at the final discretion of treating clinicians.

Outcomes

The composite primary outcome was organ support–free days evaluated on an ordinal scale that combined in-hospital death (assigned a value of −1) and, for those who survived to hospital discharge, the number of days free of respiratory or cardiovascular organ support up to day 21 after randomization (Table S1). A patient who was already in the intensive care unit and on organ support at the time of randomization would have that day and all subsequent days within the first 21 days when they were receiving organ support counted as days on which they were still on organ support (Table S1). Whenever they were taken off organ support, they began accruing organ support–free days for the remainder of the time (unless they went back on organ support or died within 21 days from their index hospital stay after being taken off organ support). If the patient was discharged alive from the hospital, they were no longer on organ support and therefore considered organ support–free for any days from the discharge date through day 21. For those participants who went off organ support and went back on, the organ support–free days score was computed from the first time they went on until the last time they went off organ support through day 21. All days after discharge were organ support–free days.

Any death during the index hospitalization through 90 days was assigned the worst outcome (−1). This outcome reflects both use of critical care therapies and survival with a range of −1 to 21 days; higher values indicate less organ support and better outcomes; an odds ratio (OR) >1 indicated treatment benefit. We also evaluated the proportion of patients with in-hospital death through day 90 and those with death during the 90 days after randomization (in or out of the hospital). Patients who were discharged from the hospital alive but died later are included in the results for death through day 90 but are not assigned a value of death for the primary end point.

Key secondary outcomes included the composite of major thrombotic events (pulmonary embolism, systemic arterial thromboembolism, myocardial infarction, or ischemic stroke) or death during hospitalization or at 28 days after enrollment, and days free of death, respiratory and cardiovascular organ support, and renal replacement therapy during the index hospitalization through day 28. Prespecified safety outcomes were all-cause death, deep vein thrombosis, pulmonary embolism, myocardial infarction, ischemic stroke, other arterial or venous thromboembolism events, symptomatic intracranial or intracerebral hemorrhage, major bleeding at 28 days according to the definition of the International Society on Thrombosis and Hemostasis, infusion-related serious adverse events (eg, anaphylaxis), and pregnancy.

All reported bleeding and thrombotic events were adjudicated according to prespecified consensus definitions by a clinical end-point committee whose members were unaware of trial group assignments (Supplemental Appendix). A comprehensive list of study outcomes is provided in the trial protocol and the statistical analysis plan (Supplemental Appendix).

Statistical Analysis

The adaptive design provided a flexible sample size, with prospective interim analyses conducted by an independent statistical analysis committee every time 200 participants completed the follow-up assessment of the primary end point. Prespecified statistical thresholds for superiority (>99% posterior probability of a proportional OR >1, implying improved outcomes on organ support–free days) and futility (>95% posterior probability of a proportional OR <1.2), considering organ support–free days and all-cause death, were separately assessed for the moderate and severe illness cohorts at each interim and final analysis. Randomization continued until a statistical threshold for the declaration of superiority or futility as a conclusion was met.

We included data from all enrolled patients with confirmed COVID-19 randomized to either crizanlizumab plus standard of care or standard of care alone in the analysis for the primary and secondary outcomes, according to the intention-to-treat principle. All controls were concurrent; no patients who were randomized before the crizanlizumab domain began enrollment were included in the control group for this analysis, and there was no difference in dates of recruitment. Baseline characteristics were summarized across arms as means and SDs, medians

and interquartile ranges, or percentages. The primary analysis model was a hierarchical Bayesian cumulative logistic model for the ordinal primary end point, estimating separate proportional ORs for each cohort (moderate and severe illness), with dynamic borrowing between the cohorts to inform each treatment effect. The primary model was adjusted for age, sex, hospital site country, history of cardiovascular disease (composite of hypertension, heart failure, coronary artery disease, peripheral artery disease, and cerebrovascular disease), other randomly assigned treatments (sodium-glucose cotransporter-2 inhibitors) within ACTIV-4a, illness severity, respiratory support at enrollment, and enrollment time (in 4-week intervals). The assumption of proportional treatment effects across the scale of the ordinal outcome was not tested due to small probabilities (<5%) for the tails of the ordinal end point. A Markov chain Monte Carlo algorithm with 100 000 samples from the joint posterior distribution was used to fit the primary analysis model to account for a weakly informative Dirichlet prior distribution for the organ support–free days outcome.

Similar Bayesian cumulative logistic models including a flat Dirichlet prior were used to examine the components of the primary outcome. The consistency of the treatment effect on the outcome of organ support–free days and its components was further examined by frequentist logistic models.

Secondary and subgroup analyses were conducted by frequentist and cumulative logistic models. Binary outcomes were estimated through random-effects logistic regression models adjusted for the same covariates as the primary analysis model. Time remaining alive and time remaining alive and free of organ support (as post hoc analysis) were assessed by frailty proportional hazards models adjusted for the same covariates as the primary analysis model. Proportional hazards assumptions for the secondary outcomes were tested by comparison of survival curves and log[log] curves by treatment and by including parameters for covariate×log(time) interaction. For subgroup analysis testing, treatment×group interactions were included in the models. Both secondary and subgroup analyses not using a Bayesian approach were not adjusted for type I error related to multiple comparisons.

Analyses were performed with R version 4.1 (R Foundation for Statistical Computing) for Bayesian models and SAS version 9.4 (SAS Institute Inc) for frequentist models. Additional information on the statistical analysis is detailed in the statistical analysis plan (Supplemental Appendix).

RESULTS

Enrollment

The first patient was randomized to the crizanlizumab domain on December 9, 2021. Enrollment was stopped on September 23, 2022, after a planned interim analysis including data through August 29, 2022, demonstrated that the prespecified statistical criterion for futility had been met with data from 383 randomized patients (339 patients with moderate COVID-19 and 44 patients with severe COVID-19). The final analysis population consisted of 422 patients, of whom 211 (50%) were randomized to crizanlizumab plus standard of care and 211 (50%) were randomized to standard of care alone (Figure 1). Of patients randomized to crizanlizumab, 198 (94%) received the study drug, which was administered as a single infusion.

Patients

The mean age of randomized patients was 68 years (SD, 13.5 years); 40% were female; and 14% reported Hispanic ethnicity (Table 1). Of patients with available data on race (n=384), 60% were White, 26% were Black, and 7% were Asian. Patients had high rates of baseline cardiometabolic risk factors, including hypertension (n=306; 73%), diabetes (n=193; 46%), and heart failure (n=123; 29%). Baseline therapies before randomization included remdesivir (76%), corticosteroids (70%), baricitinib (5%), and interleukin-6 antagonists (2%). Before randomization, among patients with known anticoagulation status (n=323), 147 patients (46%) received prophylactic-dose heparin, 86 (27%) received therapeutic-dose heparin, and 90 (28%) received no anticoagulation.

The majority of randomized patients (n=373; 186 randomized to crizanlizumab plus standard of care and 187 randomized to standard of care alone) had moderate illness at the time of randomization, whereas 49 patients (25 randomized to crizanlizumab plus standard of care and 24 randomized to standard of care alone) had severe illness. Randomized groups were generally well balanced across most baseline characteristics in the overall population (Table 1) and by moderate or severe illness (Tables S2 and S3). Of note, among all patients with available C-reactive protein at or near the time of randomization (n=208), C-reactive protein was higher among patients randomized to crizanlizumab plus standard of care (median, 78.9 mg/L; interquartile range, 39.4–139.0 mg/L) compared with those randomized to standard of care alone (median, 46.8 mg/L; interquartile range, 22.0–130.0 mg/L).

Primary Outcomes and Components

Among 421 randomized patients with known 21-day outcomes, the majority (n=332; 79%) did not require respiratory or cardiovascular organ support and therefore had 21 organ support–free days (Figure 2). A total of 163 patients (77%) randomized to the crizanlizumab plus standard-of-care group did not require any respiratory or cardiovascular organ support through day 21 compared with 169 (80%) in the standard care alone group. This corresponded to an adjusted posterior median OR for the effect of crizanlizumab on organ support–free days of 0.70 (95% CI, 0.43–1.16; posterior probability of futility, 98%; posterior probability of inferiority, 91%; Table 2; Figure 2). A total of 37 patients (17.5%) in the crizanlizumab plus standard-of-care group and 27 patients (12.9%) in the standard care alone group died during the first 90 days after

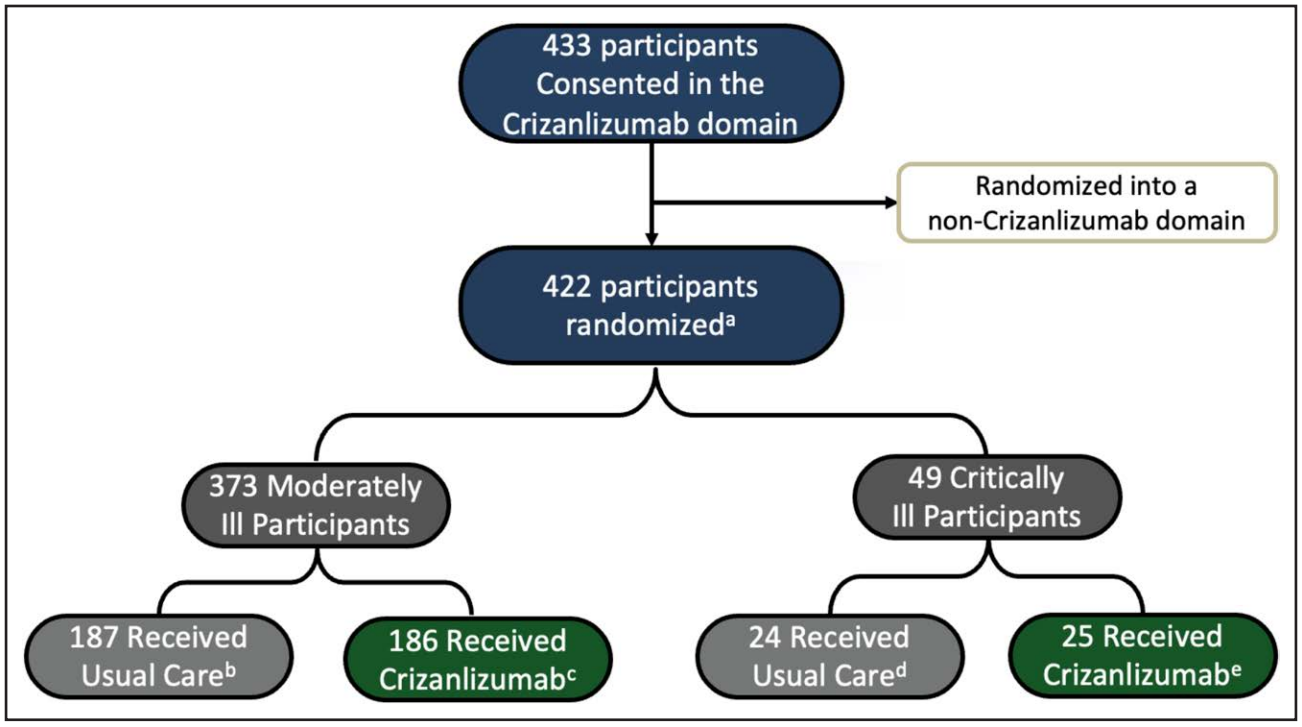

Figure 1. Eligibility and randomization in the ACTIV-4a trial of crizanlizumab in patients hospitalized for COVID-19.
[a] Randomization stratified by site and by illness severity. [b]A total of 187 participants were included in the organ support–free days [OSFD]) end-point assessment. [c]A total of 186 participants were included in the OSFD end point assessment. [d]Twenty-three participants were included in the OSFD end-point assessment. [e]Twenty-five participants were included in the OSFD end-point assessment. ACTIV-4a indicates Accelerating COVID-19 Therapeutic Interventions and Vaccines 4 ACUTE.

randomization (hazard ratio [HR], 1.33 [95% CI, 0.85–2.21]; HR >1=0.879; Figure 3).

Among patients with moderate illness (n=373), 161 (87%) randomized to the crizanlizumab plus standard-of-care group and 167 (89%) randomized to the standard-of-care–alone group did not require any respiratory of cardiovascular organ support through day 21. The posterior median OR for the effect of crizanlizumab on organ support–free days among patients with moderate illness was 0.82 (95% CI, 0.43–1.55; posterior probability of futility, 88%; posterior probability of inferiority, 75%). Twenty-five patients (13.4%) with moderate illness in the crizanlizumab plus standard care group and 20 patients (10.7%) in the standard care alone group died during the first 90 days after randomization (HR, 1.30 [95% CI, 0.74–2.27]; HR >1=0.816).

Similar trends were observed in the smaller sample of patients with severe illness (n=48). The posterior median OR for the effect of crizanlizumab on organ support–free days among patients with severe illness was 0.96 (95% CI, 0.37–2.75; posterior probability of futility, 68%; posterior probability of inferiority, 54%). Twelve patients (48.0%) with severe disease in the crizanlizumab group and 7 patients (30.4%) in the standard-of-care–alone group died during the first 90 days after randomization (HR, 1.61 [95% CI, 0.72–4.21; HR >1=0.862).

A frequentist cumulative logistic model showed results similar to those of the Bayesian analysis (Table S4). Results were similar across prespecified subgroups in adjusted intention-to-treat analyses (Table S5) and in an as-treated analysis (Table S6).

Secondary and Safety Outcomes

Overall, thrombotic events occurred more frequently in the standard care arm (Table 3), with pulmonary embolism occurring in 0 patients in the crizanlizumab plus standard-of-care arm and 4 patients in the standard-of-care arm, deep venous thrombosis occurring in 3 patients in the crizanlizumab plus standard-of-care arm and 6 in the standard-of-care arm, other arterial thromboembolic events occurring in 0 patients in the crizanlizumab plus standard-of-care arm and 1 patient in the standard-of-care arm, and myocardial infarction occurring in 1 patient in the crizanlizumab plus standard-of-care arm and 0 patients in the standard-of-care arm.

The primary safety outcome of major bleeding occurred in 6 patients (2.8%) in the crizanlizumab plus standard care arm and in 4 patients (1.9%) in the standard care alone arm (adjusted OR, 1.29 [95% CI, 0.33–4.99]; *P*=0.52]). Additional secondary outcomes are listed in Table 3.

ORIGINAL RESEARCH ARTICLE

Table 1. Baseline Participant Characteristics

Measure	Crizanlizumab (n=211)	Standard care (n=211)
Age, mean±SD, y	67.5±13.9	68.7±13.3
Sex, n (%)		
Female	80 (37.9)	90 (42.7)
Male	131 (62.1)	121 (57.3)
Race, n (%)*	(n=191)	(n=193)
American Indian or Alaska Native	1 (0.5)	0 (0.0)
Asian	15 (7.9)	12 (6.2)
Black	50 (26.2)	48 (24.9)
Multiple races	1 (0.5)	1 (0.5)
Native Hawaiian or Pacific Islander	0 (0.0)	1 (0.5)
White	111 (58.1)	120 (62.2)
Other	13 (6.8)	11 (5.7)
Hispanic, n/N (%)	31/204 (15.2)	25/204 (12.3)
Body mass index, median (n) [Q1–Q3], kg/m^2	27.0 (205) [23.0–33.8]	29.7 (210) [24.4–34.9]
Body mass index >30 kg/m^2, n/N (%)	76/205 (37.1)	101/210 (48.1)
Cardiovascular disease, n (%)	179 (84.8)	165 (78.2)
Hypertension	156 (73.9)	150 (71.1)
Heart failure	64 (30.3)	59 (28.0)
Coronary artery disease	52 (24.6)	61 (28.9)
Peripheral arterial disease	13 (6.2)	11 (5.2)
Cerebrovascular disease	29 (13.7)	21 (10.0)
Other diseases and chronic conditions, n/N (%)†	126/209 (60.3)	127/210 (60.5)
Diabetes	94/209 (45.0)	99/210 (47.1)
Chronic kidney disease	64/209 (30.6)	67/210 (31.9)
Liver disease	6/209 (2.9)	12/210 (5.7)
Respiratory disease, n (%)†‡	60 (28.4)	84 (39.8)
Asthma	30 (14.2)	32 (15.2)
COPD	42 (19.9)	63 (29.9)
Baseline treatment, n (%)		
Steroids	155 (73.5)	142 (67.3)
Anticoagulant	(n=166)	(n=157)
None	46 (27.7)	44 (28.0)
Prophylactic	73 (44.0)	74 (47.1)
Therapeutic	47 (28.3)	39 (24.8)
Remdesivir, n/N (%)	161 (76.3)	160/210 (76.2)
Aspirin	100 (47.4)	93 (44.1)
Baricitinib	13 (6.2)	10 (4.7)
IL-6 inhibitors	5 (2.4)	2 (0.9)
Organ support, n (%)		
Respiratory support	(n=135)	(n=128)
None, nasal cannula, venturi mask	109 (80.7)	103 (80.5)
High-flow nasal cannula (>20 L/min)	14 (10.4)	20 (15.6)
Noninvasive ventilation	5 (3.7)	2 (1.6)
Invasive mechanical ventilation	7 (5.2)	3 (2.3)
Vasopressors or inotropes	4 (1.9)	3 (1.4)
Laboratory values, median [Q1–Q3]§		
D-dimer, µg/L (FEU)	1187 [691–2354] (112)	1178 [665–2356] (132)
≥2-fold the ULN, n/N (%)	65/112 (58.0)	75/132 (56.8)
C-reactive protein, mg/L	78.9 [39.4–139] (111)	46.8 [22.0–130] (97)
Creatinine, mg/dL	1.09 [0.81–1.70] (210)	1.10 [0.80–1.78] (210)
Hemoglobin, g/dL	11.8 [9.80–13.2]	12.0 [10.3–13.5] (209)
Lymphocyte count, 10^9/L	0.90 [0.50–1.37] (158)	0.88 [0.50–1.41] (160)
Platelet count, 10^3/µL	199 [146–249]	193 [140–255] (208)
Troponin I, ng/mL	0.03 [0.01–0.07] (59)	0.03 [0.01–0.12] (53)

(*Continued*)

COPD indicates chronic obstructive pulmonary disease; FEU, fibrinogen equivalent units; IL-6, interleukin-6; and ULN, upper limit of normal.

*This trial collected self-reported race and ethnicity data from either the participants or their surrogates through fixed categories appropriate to their region.

†Data were systematically collected for each subject using predefined questions in the electronic case report form. If this information was not available or was not reported in the hospital records, the site personnel ticked either "not reported" or "information not available" (as appropriate).

‡Includes home oxygen therapy, asthma, COPD, bronchiectasis, interstitial lung disease, lung cancer, pulmonary hypertension, and tuberculosis.

§Each site provided the local normal ranges for the assays: C-reactive protein, creatinine, hemoglobin, lymphocyte count, and platelet count. The normal ranges differed per site. For the analysis, all assay results and units were standardized and converted to SI standards.

DISCUSSION

In this open-label, international, multicenter, randomized controlled platform trial of hospitalized patients with moderate to severe COVID-19, we found that the P-selectin inhibitor crizanlizumab plus standard of care did not reduce the primary outcome of the number of organ support–free days compared with standard of care alone. There was no significant difference between groups receiving crizanlizumab plus standard of care or standard of care for any prespecified secondary end points, including deep vein thrombosis, pulmonary embolism, and myocardial infarction, although the number of these events was low. Results were similar for prespecified subgroups, including patients with severe COVID-19, patients with moderate COVID-19, sex, race, and obesity.

COVID-19 is characterized by vascular injury, demonstrated by histological evidence of microvascular inflammation and thrombosis and by elevated levels of vasoactive molecules released by activated endothelial

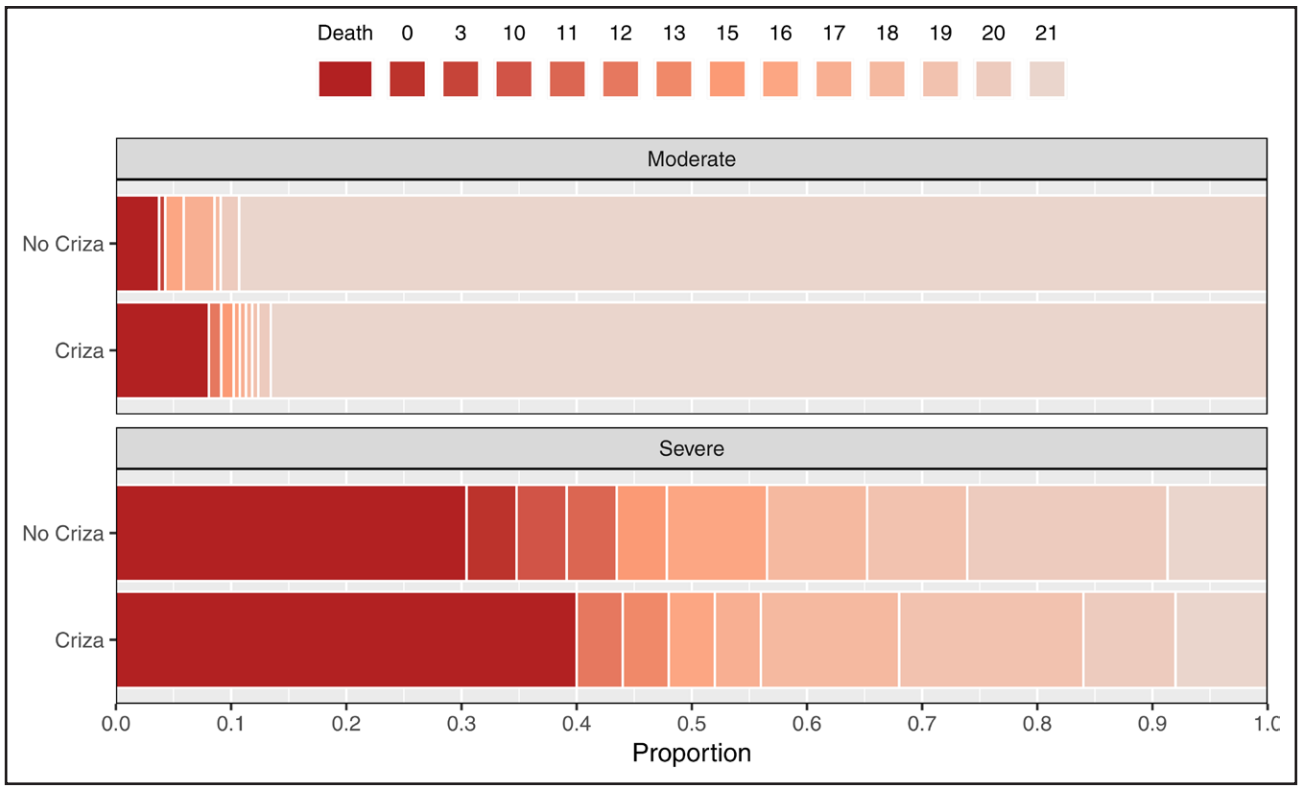

Figure 2. Effect of randomization to crizanlizumab plus standard of care vs standard of care alone on the number of days not requiring respiratory or cardiovascular organ support.
Criza indicates crizanlizumab.

cells such as P-selectin and von Willebrand factor.[1–3] These mechanisms have been postulated to play a role in both the macrovascular and microvascular complications seen in severe forms of the disease. Nevertheless, whether these endothelial biomarkers mediate inflammation and thrombosis or merely reflect vascular damage is unclear. P-selectin plays a central role in vascular inflammation, mediating leukocyte adhesion to the vessel wall[11] and promoting thrombosis by stabilizing platelet aggregation.[12] Animal studies support the concept that P-selectin may directly mediate inflammation and thrombosis. For example, genetic deletion of P-selectin limits vascular inflammation and prolongs survival in mice with sepsis.[13,14] P-selectin inhibitors or genetic targeting of P-selectin also decreases thrombosis in mice.[15,16] That P-selectin levels are elevated in patients with COVID-19 and that targeting P-selectin decreases inflammation and thrombosis in experimental settings provided the rationale for testing a P-selectin inhibitor in COVID-19.

We previously found that crizanlizumab at the dose and frequency studied in ACTIV-4a lowered soluble P-selectin levels by 89% in patients hospitalized for COVID-19 and was associated with several biomarker changes suggestive of increased fibrinolysis,[8] including the combination of elevated D-dimer and prothrombin fragment 1.2. Thus, we hypothesized that in a larger trial, blocking P-selectin might lead to improvement in outcomes in patients hospitalized for COVID-19. However, in this trial, we observed no improvement in organ support–free survival, with deaths occurring more frequently in the crizanlizumab plus standard-of-care arm. Thrombotic events were numerically lower in those receiving crizanlizumab plus standard of care, and major bleeding was infrequent and similar; the overall number of events for both of these outcomes was low. It is possible that crizanlizumab may have reduced thrombotic events without causing excess bleeding while failing to improve the more common COVID-19–related respiratory complications leading to death.

There are several potential interpretations for the neutral results observed in this trial. The most direct explanation is that P-selectin is a marker for endothelial activation but does not mediate the predominant pathways leading to severe complications and death in COVID-19. Another possibility is that because P-selectin is one of several selectins that mediate vascular inflammation in COVID-19, inhibition of P-selectin alone is insufficient to decrease inflammation; this is supported by mouse studies showing that mice lacking E-selectin, L-selectin, and P-selectin have much less vascular inflammation than mice lacking P-selectin alone.[17] An alternative interpretation is that P-selectin may mediate the early stages of vascular inflammation, whereas other adhesion molecules control the later stages, a finding observed in

Table 2. Primary Outcome and Individual Components

Outcome	Crizanlizumab	Standard care	Absolute difference (95% CI)	Median adjusted OR/HR (95% CrI)*†	Probabilities		
					Futility	Superiority	Inferiority
Overall, n	211	210‡					
Organ support–free days (primary end point), median (IQR)§‖	21 (21, 21)	21 (21, 21)	…	0.70 (0.43, 1.16)	0.983	0.093	0.907
In-hospital death, n (%)	25 (11.8)	14 (6.7)	5.2 (−0.4, 10.7)	1.89 (0.93, 3.85)	0.990	0.036	0.964
Death through d 90, n (%)	37 (17.5)	27 (12.9)	4.7 (−2.2, 11.6)	1.33 (0.85, 2.21)	0.979	0.121	0.879
Moderate, n	186	187					
Organ support–free days (primary end point), median (IQR)§‖	21 (21, 21)	21 (21, 21)	…	0.82 (0.43, 1.55)	0.883	0.252	0.748
In-hospital death, n (%)	15 (8.1)	7 (3.7)	4.3 (0.5, 9.1)	1.82 (0.78, 4.55)	0.966	0.079	0.921
Death through d 90, n (%)	25 (13.4)	20 (10.7)	2.7 (−3.9, 9.4)	1.30 (0.74, 2.27)	0.930	0.184	0.816
Severe	25	23					
Organ support–free days (primary end point), median (IQR)§‖	16 (−1, 19)	16 (−1, 19)	…	0.96 (0.37, 2.75)	0.681	0.463	0.537
In-hospital death, n (%)	10 (40.0)	7 (30.4)	9.6 (−18.7, 37.8)	1.33 (0.41, 4.00)	0.779	0.323	0.677
Death through d 90, n (%)	12 (48.0)	7 (30.4)	17.6 (−11.0, 46.1)	1.61 (0.72, 4.21)	0.947	0.138	0.862

CrI indicates credible interval; HR, hazard ratio; IQR, interquartile range; and OR, odds ratio.

*Adjusted for age, sex, enrollment epoch, cardiovascular disease (composite of hypertension, heart failure, coronary artery disease, peripheral artery disease, and cerebrovascular disease), baseline mechanical ventilation, and site (modeled within parent country), sodium-glucose cotransporter-2 assignment (active, control, or eligible not enrolled).

†The reported effect estimate for organ support–free days is a proportional OR from a cumulative logistic regression model; for in-hospital death, it is an OR from a binary logistic regression model; and for death through day 90, it is an HR from a piecewise exponential survival model.

‡One participant randomized to receive standard of care alone withdrew early from the trial and therefore was not included in the 21-day primary outcome assessment as per the statistical analysis plan.

§Composite ordinal scale consisting of survival to hospital discharge and days free of organ support to day 21. OR >1 indicates a benefit from treatment. Probabilities of benefit (proportional OR >1 for organ support–free days end point; OR/HR <1 for mortality end points), inferiority (proportional OR <1 for organ support–free days end point; OR/HR >1 for mortality end points), and futility (proportional OR <1.2 for organ support–free days end point; OR/HR <0.833 for mortality end points) are computed from the posterior distribution of the proportional odds ratio for crizanlizumab compared with no crizanlizumab on the outcome. Note that a higher OR for the organ support–free days end point represents a better outcome (more organ-support free days); however, a lower OR/HR for the mortality end points represents a better outcome (lower risk of death).

‖The model incorporated dynamic borrowing between critically ill and non–critically ill participants. The mean treatment effect in each group was assumed to follow a hierarchical normal distribution with the same mean, which created a dynamic amount of borrowing, depending on the similarity across groups. When consistent effects were observed for the groups, the posterior distribution for each intervention group effect was shrunk toward the overall estimate.

mouse models, limiting the benefit of P-selectin inhibition alone in patients with well-established inflammation days after COVID-19 infection.[18] Although P-selectin plays a role in the recruitment of leukocytes to an injury site during inflammation, raising a theoretic concern about an increased risk of infection associated with P-selectin

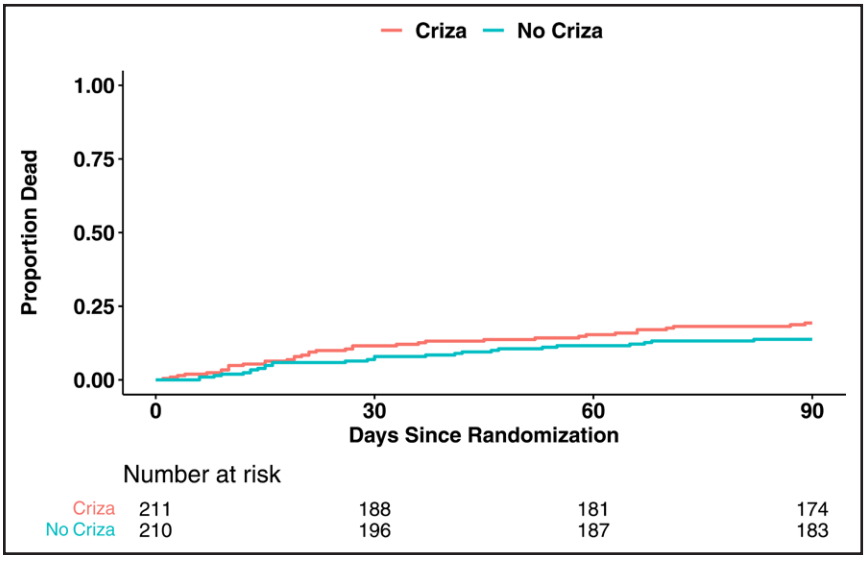

Figure 3. Effect of randomization to crizanlizumab plus standard of care vs standard of care alone on time to death in patients hospitalized for COVID-19.
Criza indicates crizanlizumab.

Table 3. Secondary Outcomes

28-d outcome*	Crizanlizumab (n=211)	Standard care (n=211)
Days free of organ support (n)	22.6±11.0 (210)	23.6±9.91 (205)
Any renal replacement therapy, n/N (%)†	6/185 (3.2)	4/191 (2.1)
Days free of renal replacement therapy (n)	25.0±8.58 (185)	25.9±7.41 (191)
Days free of organ support and renal replacement therapy (n)	24.4±9.00 (185)	25.1±7.90 (191)
Days free of respiratory support (n)‡	24.3±8.96 (210)	25.4±7.43 (205)
Days free of vasopressors (n)	24.6±9.09 (210)	25.9±7.21 (205)
Any extracorporeal membrane oxygenation, n/N (%)	0/210 (0.0)	1/205 (0.2)
Clinical progression scale		
Peak of daily scores (n)	4.09±1.69 (208)	3.87±1.45 (202)
Percentage of daily scores with improvement in ≥2 categories	67.9 [0.00–92.9] (207)	75.0 [0.00–92.9] (202)
Pulmonary embolism, n (%)	0 (0.0)	4 (1.9)
Deep vein thrombosis, n (%)	3 (1.4)	6 (2.8)
Other arterial venous thromboembolism, n (%)	0 (0.0)	1 (0.5)
Myocardial infarction, n (%)	1 (0.5)	0 (0.0)
Ischemic stroke, n (%)	0 (0.0)	0 (0.0)
Major thrombotic event or in-hospital death, n (%)§	18 (8.5)	15 (7.1)
Thrombotic event or in-hospital death, n (%)‖	20 (9.5)	19 (9.0)
Intracranial hemorrhage, n (%)	1 (0.5)	0 (0.0)
Major bleeding, n (%)¶	6 (2.8)	4 (1.9)
Bleeding causing a fall in hemoglobin level of ≥2 g/dL	0 (0.0)	2 (0.9)
Leading to transfusion of ≥2 U whole blood or red cells	2 (0.9)	1 (0.5)
Symptomatic bleeding in a critical area or organ	3 (1.4)	0 (0.0)
Fatal	1 (0.5)	1 (0.5)

*Data expressed as mean±SD or median [Q1–Q3] as appropriate.
†For those not on renal replacement therapy at baseline.
‡High-flow nasal cannula, noninvasive/invasive ventilation, or extracorporeal membrane oxygenation.
§Major thrombotic events include pulmonary embolism, myocardial infarction, ischemic stroke, and other arterial or venous thromboembolism events.
‖Thrombotic events include major thrombotic events and deep vein thrombosis.
¶Major bleeding events are defined accordingly by the International Society on Thrombosis and Hemostasis: fatal bleeding; symptomatic bleeding in a critical area or organ such as intracranial, intraspinal, intraocular, retroperitoneal, intraarticular or pericardial, or intramuscular with compartment syndrome; or bleeding causing a fall in hemoglobin level of ≥2 g/dL or leading to transfusion of ≥2 U whole blood or red cells.

inhibition, in a randomized trial of crizanlizumab for sickle cell disease, infection-related adverse events were similar between treatment arms.[7] The overall lower severity of disease, especially in moderately ill patients, who made up the majority of patients enrolled in this trial, possibly due to emergence of less virulent SARS-CoV-2 variants, higher community vaccination rates, and higher natural immunity to the virus, may have contributed to an overall low risk of adverse events. Finally, the low number of thrombotic events in the present cohort renders this analysis underpowered to independently assess the impact of P-selectin inhibition on thrombosis.

Limitations

Several limitations of this study should be noted. Although the trial was stopped for reaching the prespecified futility margin in the moderate illness group, with no suggestion of benefit in the severe group, the total number of outcomes, including deaths and progression to organ support, was low. Although we observed a numerically higher number of deaths in the crizanlizumab plus standard-of-care arm, the overall number of deaths in this trial was small, and this observation was most apparent in the moderate illness group. Similarly, we observed no increase in nonfatal adverse events in the crizanlizumab plus standard-of-care group. These findings suggest that the numerically higher number of deaths observed in those receiving crizanlizumab is likely to be due to chance. Nevertheless, we cannot rigorously exclude the possibility that P-selectin inhibition could be harmful in hospitalized patients with COVID-19. We also cannot rule out the possibility that minor baseline imbalances between the treatment arms may have contributed to the increased number of fatal events in the crizanlizumab plus standard of care arm. Finally, although these data suggest that crizanlizumab would not benefit patients hospitalized for COVID-19, they do not inform about the approved use of crizanlizumab for prevention of sickle cell crisis.

Conclusions

Crizanlizumab, a P-selectin inhibitor, did not result in improvement in organ support–free days or in-hospital death in patients hospitalized for COVID-19 with moderate or severe illness. Although crizanlizumab has been proven safe and effective for reducing sickle cell crisis, these data do not support its use for reducing the complications in patients hospitalized for COVID-19 and suggest that inhibition of P-selectin is unlikely to benefit patients with moderate to severe COVID-19.

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Supplemental Material

ACTIV4a Investigators and Collaborators

Tables S1–S6

ACTIV-4 ACUTE (AC-INPT): Protocol Document

ACTIV-4 Statistical Analysis Plan for the Randomized Clinical Trial of Crizanlizumab: Protocol 1.2

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