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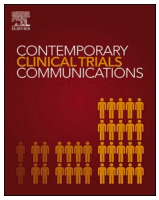

Design of VA CoronavirUs Research and Efficacy Studies-1 (VA CURES-1): A double-blind, randomized placebo-controlled trial of COVID-19 convalescent plasma in hospitalized patients with early respiratory compromise

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ABSTRACT

Background: Effective therapeutics for severe acute respiratory syndrome CoronaVirus-2 (SARS-CoV-2) infection are evolving. Under Emergency Use Authorization, COVID-19 convalescent plasma (CCP) was widely used in individuals hospitalized for COVID-19, but few randomized controlled trials supported its efficacy to limit respiratory failure or death.

Methods: VA CoronavirUs Research and Efficacy Studies-1 (VA CURES-1) was a double-blind, multi-site, placebo-controlled, randomized clinical trial evaluating the efficacy and safety of CCP with conventional therapy in hospitalized Veterans with SARS-CoV-2 infection and early respiratory compromise (requirement for oxygen). Participants (planned sample size 702) were randomized 1:1 to receive CCP with high titer neutralizing activity or 0.9% saline, stratified by site and age (≥65 versus <65 years old). Participants were followed daily during initial hospitalization and at Days 15, 22 and 28.

Outcomes: The composite primary outcome was acute hypoxemic respiratory failure or all-cause death by Day 28. Secondary outcomes by day 28 included time-to-recovery, clinical severity, mortality, rehospitalization for COVID-19, and adverse events. Serial respiratory and blood samples were collected for safety, virologic and immunologic analyses and future studies. Key variables in predicting the success of CURES-1 were: (1) enrollment early in the course of severe infection; (2) use of plasma with high neutralizing activity; (3) reliance on unambiguous, clinically meaningful outcomes. CURES-1 was terminated for futility due to perceived inability to enroll in the lull between the Alpha and Delta waves of the SARS CoV-2 epidemic.

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Conclusions: VA CURES-1 was a large multi-site trial designed to provide conclusive information about the efficacy of CCP in well-characterized patients at risk for progression of COVID-19. It utilized a rigorous study design with relevant initial timing, quality of product and outcomes.
Trial registration: ClinicalTrials.gov Identifier: NCT04539275.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapidly surpassed two earlier, highly lethal coronavirus species - SARS-CoV in 2003 and Middle East respiratory syndrome coronavirus (MERS) in 2012 - in the speed and breadth of its transmission worldwide. Although most infected individuals recover without intervention via innate and adaptive immunity, rates of hospitalization with an increased risk of respiratory failure and death, ≥20% early in the pandemic, remain severe [1]. Such severe outcomes are most prominent among older persons with comorbidities, e.g., chronic lung, cardiac and renal disease, hypertension, diabetes, obesity, and immunosuppression [2,3], conditions common among U.S. Veterans.

Before the availability of effective vaccines or other preventive measures, treatment options in serious disease were limited. Effective antiviral medications (e.g., remdesivir) were, until recently, few; they shortened time-to-recovery without decreasing mortality among hospitalized patients [4]. Corticosteroids improve mortality in moderate to severe disease [5]. Passive immune therapies using combinations of humanized monoclonal antibodies initially prevented progression of mild disease in outpatients [6] but are largely ineffective for severe disease [7] and were susceptible to evasion by variants. Medications that limit viral replication [8–11] or modulate inflammatory and immune responses (e.g., tocilizumab, baricitinib) [12] have been tested in those hospitalized for severe or critical disease, with promising but variable outcomes [8] and some adverse effects. Because novel SARS-CoV-2 variants might resist existing therapies and disseminate rapidly, multiple therapeutic strategies capable of rapid implementation are needed.

Convalescent plasma from persons recovered from SARS-CoV-2 (COVID-19 convalescent plasma, CCP) has been used to treat hospitalized individuals with complicated COVID-19. Although relatively safe and widely used, few data supported its efficacy to prevent respiratory failure and death in hospitalized patients [13–18]. With other viral infections (SARS, severe influenza, Ebola), initiating plasma therapy early in hospitalization was variably associated with improved outcomes in observational reports [19–22], but not consistently confirmed in randomized controlled trials (RCT) [22–25]. Before initiation of our study, trials evaluating CCP to treat COVID-19 reported conflicting results [13–18]. Such disparity could arise from variation in patient selection (especially illness duration and severity), in outcomes (qualitative vs. quantitative), and in investigational product potency. The initial U.S. Food and Drug Administration (FDA) Emergency Use Authorization (EUA) reflected an emerging consensus that administering high titers of neutralizing antibody soon after onset of hypoxemia yields better outcomes (August 2020).

Given the mixed results of convalescent plasma for acute viral infections, including with SARS-CoV-2, validating its efficacy for COVID-19 has important clinical implications. The World Health Organization (WHO) living guideline that CCP be used only in trials of severe and critical COVID-19 disease (https://app.magicapp.org/#/guideline/nBkO1E/section/nJB6MR. Click or tap if you trust this link.">https://app.magicapp.org/#/guideline/nBkO1E/section/nJB6MR), was recommended on December 7, 2021 and remains current. VA CoronavirUs Research and Efficacy Studies-1 (VA CURES-1) was designed and initiated between April and November 2020 to determine whether highly active CCP administered early during hospitalization prevents the meaningful clinical outcomes of respiratory failure and death. CURES-1 was the first stage in a platform trial to compare successive treatments for moderate-to-severe COVID-19 in the VA national healthcare system. Following brisk enrollment beginning in November 2020, the data safety monitoring committee recommended termination of the trial for futility due to languishing enrollment in June 2021, after decline of the Alpha wave and before emergence of the Delta wave of SARS CoV-2 infection in the USA.

2. Materials and methods

2.1. Objectives

The primary objective was to evaluate whether CCP can prevent progression to respiratory failure and death in hypoxemic patients hospitalized due to severe COVID-19. Secondary objectives evaluated safety and other efficacy outcomes, including time-to-recovery, mortality, clinical severity, and duration of hospital stay. Exploratory objectives evaluated the impact on CCP efficacy of time from symptom onset to CCP administration (≤7 vs. >7 days) and of the following baseline comorbidities (≤2 or ≥3 comorbidities): age ≥65 years, chronic lung, cardiac or renal disease, hypertension, diabetes, obesity, and immunosuppression (disease-related or pharmacological).

2.2. Design

VA CURES-1 was conceived as a Multi-Arm, Multi-Stage platform trial designed to evaluate promising treatments for Veterans hospitalized with moderate to severe COVID-19 (defined as early respiratory compromise requiring oxygen). Each stage of VA CURES-1 would consist of a double-blind RCT, with the most effective treatment arm from each stage serving as the comparator for newly identified treatments in the subsequent stage.

The first VA CURES-1 study was a double-blind comparison of high-titer CCP versus saline control. We selected CCP following a robust literature evaluation by an expert multi-disciplinary committee that ranked multiple potential but heretofore unproven therapies by pre-specified criteria (**Supplement 1**). CCP was the lead candidate based on scientific validity, operational feasibility, safety, and ability to address an established knowledge gap.

Twenty-three geographically diverse VA Medical Centers were identified to conduct VA CURES-1. The design was pragmatic, permitting concurrent therapies under EUA (other than CCP) and off-label use of medications approved for other indications. Accordingly, randomization was stratified by site to control for local differences in patient management during pandemic progression and differential expression of emerging viral variants. Two planned interim analyses permitted the study to stop early for efficacy or for futility based on the primary composite outcome of respiratory failure or death.

2.3. Trial interventions

Participants were randomized to receive: (1) ABO-matched CCP (two units, 400–600 mL total) or (2) 0.9% normal saline (NS) (500 mL total) by intravenous infusion. Rigorous investigational product blinding has been described [26]. Product infusion began within 36 h of randomization, at a rate of ≤150 mL/h, each unit to be infused over 2 h, with elapsed time between each not exceeding 12 h. When volume overload was a concern, only one unit of study product was transfused. Vital signs were measured before transfusion, 10–20 min after its start, at transfusion completion or discontinuation, and 30–60 min later.

CCP was obtained from a contracted provider (Vitalant Blood

Services; Scottsdale, AZ), which shipped plasma directly to study sites (under FDA IND #22686). Plasma was obtained from donors previously infected with SARS-CoV-2 who were symptom-free for 14–27 days with a negative nasopharyngeal swab for SARS-CoV-2 RNA or ≥28 days without one. All CCP units were guaranteed to be the upper half of antiviral activity among donations, and to show neutralizing titers of ≥1:250 by plaque reduction neutralization titer (PRNT). The neutralizing titer is the plasma dilution (or titer) yielding >50% reduction in viral plaques with live virus [the Washington strain SARS-CoV-2 (2019-nCoV/USA-WA1/2020, MN985325.1]), as tested by standardized assay at the BROAD Institute (Cambridge, MA) [27]. To permit identical ABO matching, sites were pre-stocked with ≥12 units comprising all blood types, which were to be replaced as administered.

2.4. Participant selection

Inclusion & exclusion criteria. Veterans (any sex or gender) hospitalized at participating VA Medical Centers were recruited based on symptoms suggestive of SARS-CoV-2 infection with laboratory confirmation per pre-specified eligibility criteria (Table 1).

2.5. Trial outcomes

2.5.1. Primary outcome

The primary efficacy outcome was the proportion of patients who advanced to respiratory failure or death from any cause by Day 28. Respiratory failure was defined as requiring mechanical ventilation, with or without endotracheal intubations, or extra-corporeal membrane oxygenation.

2.5.2. Secondary outcomes

The key secondary efficacy outcome was time-to-recovery in days, defined as attaining stages 1–3 on the modified WHO 8-point ordinal scale (**Table 2**) by Day 28. Additional secondary efficacy outcomes included: time to death or respiratory failure by Day 28; proportion of and time to death, respiratory failure or requiring humidified heated high-flow nasal cannula at ≥ 15 Lpm by Day 28; 28-day all-cause mortality; change in clinical severity using the WHO ordinal scale and the National Early Warning Score-2 (NEWS2) [28] score over the 28-day observation period; duration of initial hospitalization; and number of hospitalizations related to COVID-19 (for full list, Supplemental Table 1).

Table 1
Subject entry requirements for VA CURES-1 at time of randomization.

Inclusion criteria:
1) ≥ 18 years of age at time of screening;
2) agreement to provide informed consent;
3) understanding and agreement to comply with planned study procedures;
4) laboratory-confirmed SARS-CoV-2 infection as determined by polymerase chain reaction or antigen positive test ≤72 h prior to screening (if collected >72 h but ≤168 h prior to screening, documented inability to obtain a repeat sample, and progressive disease suggestive of ongoing SARS-CoV-2 infection);
5) new or increased (≥2L above baseline) oxygen requirement by nasal cannula or by facemask;
6) ability to be randomized within 72 h of hospital admission;
7) no participation in another therapeutic clinical trial for treatment of COVID-19 or SARS-CoV-2 through Day 29 without investigator approval.

Exclusion criteria:
1) Requirement for humidified heated high-flow nasal cannula (HHHFNC) at ≥ 15 Lpm
2) respiratory failure requiring mechanical ventilation, non-invasive ventilation including CPAP (for an indication other than previously diagnosed sleep apnea and maintained on outpatient settings), or extra-corporeal membrane oxygenation or anticipated to require any of those treatments or to die within 24 h;
3) anticipated discharge from the hospital or transfer to another-study site hospital within 72 h;
4) history of previous transfusion reaction;
5) previously documented serum IgA deficiency (<7 mg/dL);
6) documented to have received CCP in the last 60 days.

Table 2
Modified WHO 8-point ordinal scale for clinical improvement.

Patient State	Descriptor	Clinical Score
Ambulatory	No limitation of activity	1
	Limitation of activity and/or home oxygen	2
Hospitalized Mild disease	Hospitalized, no oxygen therapy	3
	Oxygen by mask or nasal prong	4
Hospitalized Severe disease	Humidified high-flow oxygen	5a
	Non-invasive ventilation	5b
	Intubation and mechanical Ventilation	6
	Ventilation + additional organ support—pressors, RRT, ECMO	7
Dead	Death	8

2.5.3. Safety outcomes

Safety outcomes included: (1) all adverse events resulting in a serious outcome; (2) treatment-related adverse events of any severity: transfusion reaction (fever, rash), transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), transfusion-related infections; (3) adverse events that have a grade 3 severity on the Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE v5.0) scale (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm), including SARS-CoV-2 related adverse events; and; (4) pre-specified temporal changes in routine laboratory indices. We collected adverse events from time of randomization until loss to follow-up, early withdrawal, or final visit completion, whichever occurred first. Discontinuation or temporary suspension of product administration was also reported.

2.5.4. Recruitment and consent

In keeping with the principle that recruitment efforts are best supported by an interdisciplinary approach [26], regular dialogue with ward staff and directed in-services was strongly encouraged. Study personnel delegated to obtain informed consent were required to train on the protocol and to obtain consent according to all applicable federal and VA policies. VA Research follows FDA Guidance on the Conduct of Clinical trials of Medical Products During the COVID-19 Public Health Emergency (https://www.fda.gov/media/136238/download).

Informed consent was obtained from study participants or, if they were unable to provide written consent, from their legally authorized representative. Flexibility in consent processes was necessary due to local COVID-19 infection control measures and guidelines that limited or encouraged avoiding unnecessary direct interpersonal contact. We allowed phone consent with signed hard copy, decontamination of paper consent forms and electronic consent. If local policies did not allow the contaminated consent form to leave the participant's hospital room, photographing every page and destroying the original was permitted. Even when electronic capture of signed consent documents (digital images and use of iMedConsent or DocuSign) was used, a separate signed hard copy of HIPAA forms was required per VA policy.

2.6. Randomization

After consent was obtained, participants were randomized within 72 h of initial hospital admission in a 1:1 ratio to receive CCP or NS, using permuted blocks of various sizes and stratified by age (≥65 vs. <65 years old) within each participating site. Randomization was conducted using an Interactive Web Response System (IWRS) developed by the Cooperative Studies Program Clinical Research Pharmacy Coordinating Center in Albuquerque, NM, and accessible only to authorized study personnel. IWRS allowed real-time randomization by blinded study site personnel. A blinded randomization certificate generated by IWRS was distributed to the unblinded local research pharmacist, who obtained

the unblinded assignment from IWRS and communicated with blood bank staff who oversee investigational product preparation, inventory management, and distribution 27.

2.7. Blinding of study interventions

To ensure an efficient, consistent double-blinding process and an optimal workflow, we developed detailed procedures for treatment assignment and product administration, uniquely requiring collaboration between the pharmacy and blood bank for the plasma and blinding, as well as nursing and the study team [26]. Interventions (CCP or NS) were physically blinded during transfusion using an opaque IV bag cover and IV tubing covers. Treatment assignment was blinded to participants, local site investigators, clinical staff, site coordinators, and study team members involved in study management. Study interventions were also blinded in the electronic medical records (EMR), electronic data capture system and IWRS except for designated unblinded personnel. The blind could not be broken to select post-study pharmacologic treatment for COVID-19 by clinical providers but could be accessed by the research pharmacist for emergency medical necessity.

2.8. Methods of data collection and duration of follow-up

We followed study participants daily during initial hospitalization, at hospital discharge, and at Study Days 15, 22 and 28 (Table 3). Virtual visits by telephone or telehealth were allowed on Day 22 (and for days 15 and 28 if necessary for clinical or quarantine requirements). Most data were obtained as part of routine clinical care and retrieved from the EMR. When feasible, study-specific specimens were obtained coincident with routine laboratory specimens to minimize unnecessary procedures and prolonged contagious exposures to SARS-CoV2.

The study data were collected and managed using DataFax version 2016, by DF/Net Research which allows sites to enter data directly into the database and access and address data queries online.

2.9. Monitoring and regulatory oversight

Consent process and adherence to study protocol and good clinical practice were monitored by the VA Site Monitoring, Auditing and Resource Team (SMART). A unique feature of SMART is its capacity to review materials remotely, directly from the EMR in real-time. Initial enrollees at each site were reviewed within 5 days of enrollment, permitting early identification of protocol deviations and timely error corrections. This process minimized cumulative deviations and informed the need for additional training and potential protocol modifications.

An independent, unblinded Data Monitoring Committee (DMC) provided independent review and monitoring of patient safety, study progress and benefit-risk assessment. The DMC met for planned interim analyses and at least every 6 months. The DMC made recommendations to the Sponsor about whether the study should continue or be stopped.

2.10. Statistical analysis plan

2.10.1. Populations for analyses

The planned primary efficacy analysis was based on the full analysis set under the intent-to-treat principle, including all participants randomized. The safety analysis was based on a modified intent-to-treat population consisting of all participants who received at least one dose of any study product.

2.10.2. Sample size & power calculations

The sample size was calculated to provide adequate power for the primary outcome (proportion of death or respiratory failure up to and including Day 28). We estimated that 702 participants would provide 85% power to detect a 10% absolute reduction when the proportion was 30% in the NS group, assuming 5% of participants have missing primary outcome data (e.g., due to loss of follow-up or early withdrawal; Table 4). For the key secondary outcome time-to-recovery, 702 participants would provide 83% power to detect a recovery rate ratio of 1.3 if approximately 70% of participants recovered by Day 28.

2.10.3. Interim analysis

The unblinded biostatistician was to conduct two interim analyses of the primary outcome at 33% and 67% of total information for DMC consideration to allow early study termination for efficacy (sufficient evidence of a benefit of CCP) or for futility (significant benefit of CCP is unlikely). We planned the ρ-family alpha spending function of $\rho = 2.5$ with a one-sided significance level 0.025 as a non-binding guide for efficacy stopping. The type I error spent at the interim efficacy analyses

Table 4
Power of a study with sample size 702 to detect differences in the primary outcome, using the z-test with a two-sided significance level 0.05 and assuming 5% missing data.

Proportion in the normal saline group	Absolute reduction	Corresponding proportion in the convalescent plasma group	Power
35%	11%	24%	88%
	10%	25%	81%
	9%	26%	72%
	8%	27%	61%
30%	11%	19%	91%
	10%	**20%**	**85%**
	9%	21%	76%
	8%	22%	66%
25%	11%	14%	95%
	10%	15%	90%
	9%	16%	83%
	8%	17%	72%

Table 3
Schedule of assessment measures.

Measure	Baseline	Product Administration	Initial Hospitalization	Discharge	Day 15	Day 22	Day 29
Demographics and Medical History	X						
Physical Exam	X						
Pregnancy test for females	X						
Admission Signs and Symptoms	X						
NEWS2 (Vital Signs)	X		X		X		X
Clinical status data	X		X		X	X	X
Adverse events	X	X	X	X	X	X	X
Concomitant medications	X	X		X	X	X	X
Safety labs	X		X		X		X
Research labs	X		X		X		X
Discharge data				X			
Readmission data					X	X	X

*Day 28 data is collected on Day 29 for complete data acquisition.

would be determined by the information time of the interim analyses according to the alpha spending function. For example, if the two interim analyses occur at exactly 33% and 67% information time, the efficacy boundaries in the z-scale are −2.947 and −2.401, respectively, and the cumulative type I error spent at the two looks are 0.002 and 0.009, respectively; the remaining type I error 0.041 to be allocated to the final analysis.

Conditional power would be used as a non-binding guide for futility stopping. Conditional power is the probability of obtaining a statistically significant treatment benefit of CCP at study completion given the data accumulated thus far and assuming a hypothesized treatment effect thereafter. If the conditional power is less than 10% under the hypothesized treatment effect in the primary outcome (30% in NS vs. 20% in CCP), consideration should be given to stopping the trial.

The non-binding characteristic for the interim efficacy and futility analyses was chosen to allow the DMC to make recommendations about early stopping based on the totality of evidence from the study and other available information external to the study.

2.11. Final statistical analysis

2.11.1. Primary outcome

The primary analysis for comparing the proportion of death or respiratory failure by Day 28 between the two treatment groups was by the Chi-square test for differences in the proportions and the 95% confidence interval (CI). We planned to perform regression analysis to adjust for randomization stratification factors (site and participant age (≥65 vs. <65 years old)) and baseline characteristics (including sex, race, Hispanic ethnicity, ABO blood type, BMI, hypertension, chronic lung, heart or kidney diseases, diabetes, immunosuppression), using logistic regression (with site as a fixed effect) or generalized linear mixed models (with site as a random effect) as appropriate. Odds ratios and 95%CI for the treatment effect were to be provided.

While precision and power could be enhanced by incorporating stratification factors in the analysis via stratification or regression, we chose a conservative unadjusted analysis since the rapidly changing dynamics of the pandemic were expected to lead variations in site-to-site sample sizes and event rates. Sensitivity analyses were planned to evaluate the impact of making different assumptions about the missing observations and use analyses methods such as multiple imputations.

2.11.2. Key secondary outcome

The primary analysis for time-to-recovery by Day 28 was an unadjusted log-rank test. Deaths on or before Day 28 would be censored at Day 29. Kaplan-Meier curves and 95%CI would be provided. If data permited, we would perform a stratified log-rank test, stratified by site and participant age (≥65 vs. <65 years). We also planned to perform Cox regression to adjust for site, age and other baseline characteristics (as for primary outcome). Recovery rate ratio and 95%CI would be provided.

2.11.3. Other secondary outcomes

Comparisons of binary outcomes (e.g., 28-day mortality) would be summarized by differences in proportions with 95%CI. Ordinal scales (e.g., clinical status) would be summarized by proportions in the categories and compared using proportional odds models. Time-to-event endpoints (e.g., time to respiratory failure or death) would be analyzed using log-rank tests, Kaplan-Meier curves, and Cox proportional hazards model. Hazard ratios (for time to an adverse outcome) or improvement rate ratios (for time to improvement) and 95%CI would be calculated. Categorical outcomes would be summarized by proportions in the categories and compared using Chi-square tests. Duration of initial hospitalization would be summarized by median days with quartiles and compared by the Wilcoxon test. Analyses of secondary outcomes would not be adjusted for multiple comparisons due to the descriptive and supportive nature of these analyses.

2.11.4. Subgroup analyses

We planned to evaluate the treatment effect on the primary outcome across the following subgroups: (1) received study product at ≤7 days vs. >7 days after symptom onset; (2) had ≤2 vs. ≥3 comorbidities at baseline (age ≥65 years, underlying chronic lung, heart, kidney disease, hypertension, diabetes, obesity or immunosuppression); (3) age <65 vs. ≥65 years at enrollment; (4) baseline BMI <35 vs. ≥35. A forest plot would display point estimates and 95%CI across subgroups. Interaction tests would be conducted to determine whether the treatment effect varies by subgroup. Subgroup analyses would not be adjusted for multiple comparisons due to the supportive nature of these analyses.

2.11.5. Estimated trial duration

Based on projected enrollment rates (two randomizations per month per site at 25 sites), we anticipated that the target sample size of 702 could be achieved by 15 months and the trial completed within 18 months. The trial might be stopped early for efficacy or futility before this sample size is attained.

2.11.6. Respiratory specimens and blood samples for translational research

VA CURES-1 included the collection of research respiratory samples and blood samples on days 1, 2 (blood only), 4, 7 in hospital and on days 15 and 29 in- or outpatient. These samples were to support translational research, including results of nasal SARS-CoV-2 RNA and sequencing, antibody levels and neutralization titers, and markers of local and systemic inflammation.

3. Results

The convalescent plasma units were collected in Denver, CO and San Francisco, CA (July 27-August 28, 2020). At this time, pre-Variant of Concern (VOC) strains (lineage A and early lineage B (D614G+) strains) were predominant [29]. The plasma was administered within months (December 4, 2020–June 1, 2021), before the emergence of the Delta VOC. All units had a neutralizing titer >1:250 against the WA-1 strain in the upper half of all donated units screened (median titer 1:900; range 1:250–1:5120) (Fig. 1).

Convalescent plasma was selected as the study product in April 2020.

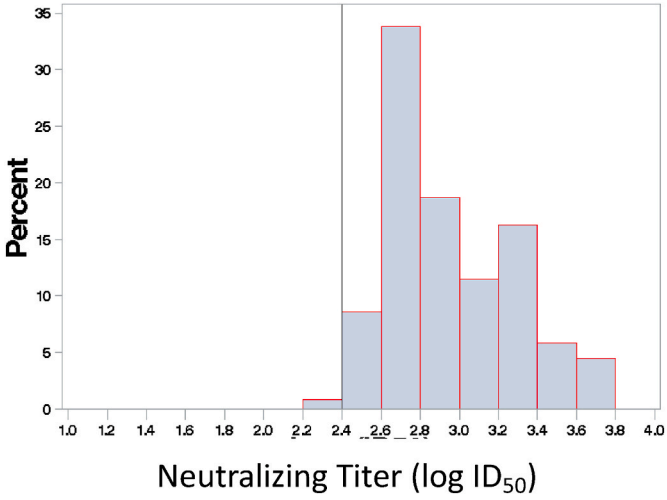

Fig. 1. Activity of Qualified Units of SARS-CoV2 Convalescent Plasma. Neutralization titers of 583 units of convalescent plasma qualified for the VA CURES-1 clinical trial. These units have a median titer of 1:900 (range 1:250–1:5120). The vertical line (log 2.4; 1:250) represents the median of all units tested by Vitalant such that all units secured for VA CURES-1 are in the upper half of neutralizing activity. Binning puts units above 1:250 in the bin to the right of the marker. The X axis represents the highest titer of antibody yielding a 50% inhibitory dose (ID50) of live SARS-CoV2 virus (neutralization cutoff), as performed at the Broad Institute [27].

Initial review of the VA CURES-1 protocol was submitted on June 15, 2020, investigational new drug approval was received by the FDA on July 29, 2020 and funding and approval by the Data Monitoring Committee (DMC) and the VA Central Institutional Review Board were completed by September 16, 200. Enrollment in VA CURES-1 began on November 19, 2020. Enrollment progressed during circulation of the alpha variant of SARS CoV-2 in late 2020 and early 2021 but declined precipitously during recession of this phase of the pandemic. In June 2020, just before the subsequent surge in cases due to the delta variant of SARS-CoV-2, the DMC recommended early termination of the study for perceived futility to accrue sufficient subjects to meet study objectives. The sponsor accepted the recommendation and accrual was terminated on June 1, 2021 after enrollment of 75 subjects. Trial status was updated in ClinicalTrials.gov Identifier: NCT04539275 and detailed clinical and laboratory results are being prepared in a forthcoming manuscript.

4. Discussion

The VA CURES-1 platform trial targeted the early phases of serious COVID-19 to determine whether interventions added to standard care reduce risk for progression to respiratory failure or death. The first trial in VA CURES-1 addressed three key variables for a robust therapeutic trial in a novel emergent infection: (1) a clearly defined target population, (2) use of optimally standardized and potent CCP, and (3) a clinically meaningful and verifiable primary outcome. The design was intended to provide unambiguous evidence for or against this therapy, initially used widely without a firm evidence base. Should benefit be proved, CCP would be added to the standard-of-care for future trials in the CURES-1 platform; if not, subsequent iterations would employ a placebo-controlled comparison of other promising agents.

Convalescent plasma was chosen as the agent for the VA CURES-1 trial during the first three months of SARS-CoV-2 spread in the United States. The study criteria and design evolved over the next 5 months based on emerging clinical reports. Initial enthusiasm for CCP was high, based on limited observational studies using convalescent plasma to treat SARS CoV-1, MERS, influenza [19,21], and early use in COVID-19. However, these studies lacked statistical power and recruited highly heterogeneous populations. Their results were of inconsistent quality due to uncontrolled [30] or unmasked designs [14,31] and endpoints of uncertain clinical relevance. Nevertheless, as CCP was one of the few plausible options at that time, and an open-label FDA-approved EUA for CCP was rapidly implemented, treating >50,000 US patients as of March 2021 [30,32]. By November 2021, Clinicaltrials.gov contained 164 clinical trials of CCP, most with similar limitations. Of the initial 52 registered at the time of the CURES-1 design, only 13 were masked RCT in adults, eight targeted a similar population to CURES-1, and only six planned to enroll ≥600 participants with sufficient numbers for adequate statistical power, 3 of which used change in the WHO ordinal scale as primary endpoint.

Early results with CCP [14,33], despite limited sample sizes, suggested that benefit was unlikely among those with respiratory failure. Thus, the CURES-1 CCP trial targeted individuals with new onset hypoxia who were not yet critically ill. The hypothesis was that early neutralization of viral replication within 72 h of admission would be efficacious.

A second consideration was antibody quality. Unlike the rigorous standards of pharmaceutical good manufacturing practices, CCP is a complex biological product, permitting only limited product standardization [26]. Levels of virus-specific antibody and neutralizing activity are essential to control acute infection [34] but vary widely following native infection [35], affected by illness severity, age, obesity, time from recovery, infection with the same or cross-reactive viral variants, medical therapy, and multiple host factors [36]. Post-hoc analyses indicate that higher levels of specific antibody were associated with better outcomes [30,37]. However, most studies published used CCP of low, inconsistent or undetermined levels of antibody or viral neutralization [38,39], confounding interpretation of clinical outcomes. The only trial treating seriously ill patients with prospective determination of neutralizing antibody included units with 50% neutralization titer >1:50 [40]. By contrast, CCP in the CURES-1 trial was from a standardized provider with relatively high viral neutralization. Because components of CCP other than antibodies [34] may influence outcome [41], we chose saline rather than non-convalescent plasma as our control, despite the increase in complexity of blinding. We will investigate the importance of specific antibodies using specimens banked in this study.

The third key element of our design was to use an objective, clinically meaningful primary outcome. Changes between WHO ordinal scale levels have disparate clinical significance, e.g., the 2-point change from level 3 (hospitalized) to level 1 (home without impairment) lacks the significance of a change from 7 (intubated with organ failure) to 5 (not intubated but hospitalized requiring oxygen). Composite endpoints with subjectively diverse clinical significance run the risk of showing a statistically significant result of uncertain clinical meaning [42]. By contrast, our primary outcome, respiratory failure or all-cause mortality, had unambiguous clinical significance to patients. However, to permit comparisons with studies using the WHO ordinal scale, we collected that data.

The VA CURES-1 CCP trial was designed and underwent peer-review between April and September of 2020, during an ascending pandemic of a highly transmissible, potentially lethal virus for which no vaccine or proven therapies were available. After conducting informed consent in person by local site investigators, we paid considerable attention to limiting exposure to study personnel, when possible, by collecting data from the EMR, study questionnaires and daily in-hospital check-ins remotely using telecommunications. Activities requiring in-person contact, including clinical examinations, administration of investigational product, and collection of study specimens were performed by licensed clinical staff trained in use of personal protective equipment.

The trial began enrollment in November of 2020, accruing rapidly during its first three months. Following the decline in COVID-19 cases that preceded the Delta variant surge in the summer of 2021, the trial was terminated in June 2021 based on the DMC's recommendation for futility to achieve enrollment goals. Had the trial continued, it is likely that recruitment targets would have been met during the rapid subsequent surge of infections the Delta variant, which led to large numbers of eligible patients at participating sites. With the benefit of hindsite, our experience supports a more conservative posture when assessing ongoing feasibility of study conduct, particular in the setting of pandemic public health emergencies. Whether the change in SARS CoV-2 strain from Alpha to Delta predominance would have affected outcomes due to a shift in viral neutralization of archived plasma is unknown. The rapid emergence of mutations leading to diminished efficacy of antiviral and monoclonal antibody therapies suggests that similar reductions in collected CCP neutralizing antibodies could have occurred.

Since study closure, two studies demonstrated a signal towards [43] or benefit [44] for disease progression in hospitalized patients. In contrast, several larger CCP RCTs, either placebo-controlled [39,45] or open label [46], did not show protection against progression or death among infected inpatients [39,45–47]. Several thoughtfully-conceived post-hoc [37] and meta-analyses [48] indicate an overall survival benefit, particularly when CCP is administered soon after infection [37] with a higher titer of virus-specific antibody [37] from geographically-proximate donors [49]. Other meta-analyses have not confirmed benefit for differences in WHO score or mortality among hospitalized patients on noninvasive supplemental oxygen [47,50]. Selected data suggest greatest benefit from CCP in hospitalized patients who are immuncompromised [46], unvaccinated [44], who have not yet developed specific antibodies, or who are not receiving other targeted therapy (e.g., remdesivir and corticosteroids) [45]. In two subsequent trials [43,44] of outpatients with COVID-19, high-quality CCP reduced

risk of hospitalization by 48–54% in mildly ill older adults, whereas two others showed no such benefit [51,52]. Contributing to this disparity of outcomes was heterogeneity among demographic, clinical and temporal variables and outcomes among those analyzed, and in plasma volume and anti-viral activity.

In summary, the VA CURES-1 platform design targeted the early phases of serious COVID-19 to determine whether interventions added to standard care reduce risk for progression to respiratory failure or death. The VA CURES design is similar to the Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV, NIH) master protocol of sequential trials that compared new agents to the most effective treatment identified in the preceding trial. VA CURES-1 most closely resembles the ACTIV-3 platform evaluating anti-SARS CoV-2 treatments for serious disease requiring hospitalization. Unlike the open label, cluster randomized RECOVERY trials (https://www.recoverytrial.net), both the VA CURES and ACTIV designs were randomized double-blinded comparisons of prospective treatments. VA CURES-1 addressed three key variables for a robust therapeutic trial in a novel emergent infection: (1) a clearly defined target population, (2) use of optimally standardized and potent CCP (for CURES-1), and (3) a clinically meaningful and verifiable primary outcome. The collection of timely sequential clinical mucosal and blood samples in this and future clinical trials should facilitate identification of mechanistic or causal correlates of disease and therapeutic outcomes.

Had anticipated variations in disease activity been acknowledged, results of this trial may have provided unambiguous evidence for or against this therapy, including against infections with evolving viral variants, initially used widely without a firm evidence base. Challenges of the CURES-1 trial were the effort to standardize a biologic product from diverse human sources, maintaining the infection control safety of staff at each stage of enrollment and follow up and maintaining consistent enrollment when faced with surges and lulls in disease activity with a heretofore unknown virus. Ultimately, enrollment was stopped when cases were at a low ebb, just prior to the even more prominent emergence of a new Delta variant. Confidence to be patient would have been rewarded. Both investigators and regulators must be aware of and make accommodation for such unpredictable disease activity as new epidemics evolve for which novel therapies must be quickly initiated and objectively evaluated.

5. Conclusions

In summary, the rigorous design of CURES-1 overcame many limitations of studies initiated early in the COVID-19 pandemic and may serve as a template for future trials should circumstances warrant. Despite early trial termination, analyses of serially collected specimens, which do not routinely accompany clinical trials, have promise to further inform the design and conduct of future trials of convalescent plasma and other SARS-COV2-specific interventions. Within the United States, the VA has a unique capacity to design and implement robust large-scale primary and collaborative clinical trials within a national healthcare system that is integrated yet geographically, ethnically and socioeconomically diverse [12,53]. Hence, maintaining the clinical infrastructure to rapidly deploy similar trials should be an important goal to support the national response to future respiratory viral pandemics and potentially other key health challenges.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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References

[1] C.C.-R. Team, Severe outcomes among patients with Coronavirus disease 2019 (COVID-19 - United States, February 12-March 16, 2020, MMWR Morb. Mortal. Wkly. Rep. (2020) 69.
[2] C.C.-R. Team, Preliminary estimates of the prevalence of selected underlying health conditions among patients with coronavirus disease 2019 - United States, february 12-march 28, 2020, MMWR Morb. Mortal. Wkly. Rep. 69 (13) (2020) 382–386.
[3] W.J. Guan, Z.Y. Ni, Y. Hu, et al., Clinical characteristics of coronavirus disease 2019 in China, N. Engl. J. Med. 382 (18) (2020) 1708–1720.
[4] J.H. Beigel, K.M. Tomashek, L.E. Dodd, et al., Remdesivir for the treatment of covid-19 - preliminary report, N. Engl. J. Med. 383 (19) (2020) 1813–1826.
[5] Group WHOREAfC-TW, J.A.C. Sterne, S. Murthy, et al., Association between administration of systemic corticosteroids and mortality among critically ill patients with COVID-19: a meta-analysis, JAMA 324 (13) (2020) 1330–1341.
[6] D.M. Weinreich, S. Sivapalasingam, T. Norton, et al., REGEN-COV antibody combination and outcomes in outpatients with covid-19, N. Engl. J. Med. 385 (23) (2021) e81.
[7] A.-T.L.-C.S. Group, J.D. Lundgren, B. Grund, et al., A neutralizing monoclonal antibody for hospitalized patients with covid-19, N. Engl. J. Med. 384 (10) (2021) 905–914.
[8] E.K. McCreary, J.M. Pogue, Coronavirus disease 2019 treatment: a review of early and emerging options, Open Forum Infect. Dis. 7 (4) (2020) ofaa105.
[9] J. Geleris, Y. Sun, J. Platt, et al., Observational study of hydroxychloroquine in hospitalized patients with covid-19, N. Engl. J. Med. 383 (25) (2020) 2411–2418.
[10] B. Cao, Y. Wang, D. Wen, et al., A trial of lopinavir-ritonavir in adults hospitalized with severe covid-19, N. Engl. J. Med. 382 (19) (2020) 1787–1799.
[11] E.J. Rubin, D.P. Harrington, J.W. Hogan, C. Gatsonis, L.R. Baden, M.B. Hamel, The urgency of care during the covid-19 pandemic - learning as we go, N. Engl. J. Med. 382 (25) (2020) 2461–2462.
[12] V.C. Marconi, A.V. Ramanan, S. de Bono, et al., Efficacy and safety of baricitinib for the treatment of hospitalised adults with COVID-19 (COV-BARRIER): a randomised, double-blind, parallel-group, placebo-controlled phase 3 trial, Lancet Respir. Med. 9 (12) (2021) 1407–1418.
[13] K. Duan, B. Liu, C. Li, et al., Effectiveness of convalescent plasma therapy in severe COVID-19 patients, Proc. Natl. Acad. Sci. U. S. A. 117 (17) (2020) 9490–9496.
[14] S.T.H. Liu, H.M. Lin, I. Baine, et al., Convalescent plasma treatment of severe COVID-19: a propensity score-matched control study, Nat. Med. 26 (11) (2020) 1708–1713.
[15] C. Shen, Z. Wang, F. Zhao, et al., Treatment of 5 critically ill patients with COVID-19 with convalescent plasma, JAMA 323 (16) (2020) 1582–1589.
[16] M. Ye, D. Fu, Y. Ren, et al., Treatment with convalescent plasma for COVID-19 patients in Wuhan, China, J. Med. Virol. 92 (10) (2020) 1890–1901.
[17] Q.L. Zeng, Z.J. Yu, J.J. Gou, et al., Effect of convalescent plasma therapy on viral shedding and survival in COVID-19 patients, J. Infect. Dis. 222 (1) (2020) 38–43.
[18] B. Zhang, S. Liu, T. Tan, et al., Treatment with convalescent plasma for critically ill patients with severe acute respiratory syndrome coronavirus 2 infection, Chest 158 (1) (2020) e3–e9.
[19] Y. Cheng, R. Wong, Y.O. Soo, et al., Use of convalescent plasma therapy in SARS patients in Hong Kong, Eur. J. Clin. Microbiol. Infect. Dis. 24 (1) (2005) 44–46.
[20] I.F.N. Hung, K.K.W. To, C.K. Lee, et al., Hyperimmune IV immunoglobulin treatment: a multicenter double-blind randomized controlled trial for patients with severe 2009 influenza A(H1N1) infection, Chest 144 (2) (2013) 464–473.

[21] J. Mair-Jenkins, M. Saavedra-Campos, J.K. Baillie, et al., The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis, J. Infect. Dis. 211 (1) (2015) 80–90.
[22] K. Mupapa, M. Massamba, K. Kibadi, et al., Treatment of Ebola hemorrhagic fever with blood transfusions from convalescent patients. International Scientific and Technical Committee, J. Infect. Dis. 179 (Suppl 1) (1999) S18–S23.
[23] J. van Griensven, T. Edwards, X. de Lamballerie, et al., Evaluation of convalescent plasma for Ebola virus disease in Guinea, N. Engl. J. Med. 374 (1) (2016) 33–42.
[24] J.H. Beigel, E. Aga, M.C. Elie-Turenne, et al., Anti-influenza immune plasma for the treatment of patients with severe influenza A: a randomised, double-blind, phase 3 trial, Lancet Respir. Med. 7 (11) (2019) 941–950.
[25] J.H. Beigel, P. Tebas, M.C. Elie-Turenne, et al., Immune plasma for the treatment of severe influenza: an open-label, multicentre, phase 2 randomised study, Lancet Respir. Med. 5 (6) (2017) 500–511.
[26] E.K. Miller, A.M. Goldberg, E.N. Janoff, et al., Designing and implementing methodology for double-blind, placebo-controlled clinical trials using blood products within the Department of Veterans Affairs, Clin. Trials (2022), 17407745211069703.
[27] S. Zhang, P. Ma, M. Orzechowski, et al., High-throughput neutralization and serology assays reveal correlated but highly variable humoral immune responses in a large population of individuals infected with SARS-CoV-2 in the US between March and august 2020, mBio 14 (2) (2023), e0352322.
[28] Physicians Rco, National Early Warning Score (NEWS) 2. Standardizing the Assessment of Acute-Illness Severity in the NHS, Updated report of a working party, 2017.
[29] P.V. Markov, M. Ghafari, M. Beer, et al., The evolution of SARS-CoV-2, Nat. Rev. Microbiol. 21 (6) (2023) 361–379.
[30] Joyner MJ, Wright RS, D'eLisa F, al e. Early safety indicators of COIVID-19 conalescent plasma in 5,000 patients. medRxiv 20020.
[31] E. Salazar, K.K. Perez, M. Ashraf, et al., Treatment of coronavirus disease 2019 (COVID-19) patients with convalescent plasma, Am. J. Pathol. 190 (8) (2020) 1680–1690.
[32] C.S. Cohn, L. Estcourt, B.J. Grossman, et al., COVID-19 convalescent plasma: interim recommendations from the AABB, Transfusion 61 (4) (2021) 1313–1323.
[33] L. Li, W. Zhang, Y. Hu, et al., Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening COVID-19: a randomized clinical trial, JAMA 324 (5) (2020) 460–470.
[34] J.D. Herman, C. Wang, C. Loos, et al., Functional convalescent plasma antibodies and pre-infusion titers shape the early severe COVID-19 immune response, Nat. Commun. 12 (1) (2021) 6853.
[35] M. Vinkenoog, M. Steenhuis, A.T. Brinke, et al., Associations between symptoms, donor characteristics and IgG antibody response in 2082 COVID-19 convalescent plasma donors, Front. Immunol. 13 (2022), 821721.
[36] P. Zimmermann, N. Curtis, Factors that influence the immune response to vaccination, Clin. Microbiol. Rev. 32 (2) (2019).
[37] M.J. Joyner, R.E. Carter, J.W. Senefeld, et al., Convalescent plasma antibody levels and the risk of death from covid-19, N. Engl. J. Med. 384 (11) (2021) 1015–1027.
[38] A. Agarwal, A. Mukherjee, G. Kumar, et al., Convalescent plasma in the management of moderate covid-19 in adults in India: open label phase II multicentre randomised controlled trial (PLACID Trial), BMJ 371 (2020) m3939.
[39] V.A. Simonovich, L.D. Burgos Pratx, P. Scibona, et al., A randomized trial of convalescent plasma in covid-19 severe pneumonia, N. Engl. J. Med. 384 (7) (2021) 619–629.
[40] W.H. Self, T.G. Stewart, A.P. Wheeler, et al., Passive Immunity Trial for Our Nation (PassITON): study protocol for a randomized placebo-control clinical trial evaluating COVID-19 convalescent plasma in hospitalized adults, Trials 22 (1) (2021) 221.
[41] D. Focosi, M. Franchini, L.A. Pirofski, et al., COVID-19 convalescent plasma is more than neutralizing antibodies: a narrative review of potential beneficial and detrimental Co-factors, Viruses 13 (8) (2021).
[42] I. Ferreira-Gonzalez, J.W. Busse, D. Heels-Ansdell, et al., Problems with use of composite end points in cardiovascular trials: systematic review of randomised controlled trials, BMJ 334 (7597) (2007) 786.
[43] R. Libster, G. Perez Marc, D. Wappner, et al., Early high-titer plasma therapy to prevent severe covid-19 in older adults, N. Engl. J. Med. 384 (7) (2021) 610–618.
[44] D.J. Sullivan, K.A. Gebo, S. Shoham, et al., Early outpatient treatment for covid-19 with convalescent plasma, N. Engl. J. Med. 386 (2022) 1700–1711.
[45] M.B. Ortigoza, H. Yoon, K.S. Goldfeld, et al., Efficacy and safety of COVID-19 convalescent plasma in hospitalized patients: a randomized clinical trial, JAMA Intern. Med. 182 (2) (2022) 115–126.
[46] Writing Committee for the R-CAPI, L.J. Estcourt, A.F. Turgeon, et al., Effect of convalescent plasma on organ support-free days in critically ill patients with COVID-19: a randomized clinical trial, JAMA 326 (17) (2021) 1690–1702.
[47] A.B. Troxel, E. Petkova, K. Goldfeld, et al., Association of convalescent plasma treatment with clinical status in patients hospitalized with COVID-19: a meta-analysis, JAMA Netw. Open 5 (1) (2022), e2147331.
[48] S.A. Klassen, J.W. Senefeld, P.W. Johnson, et al., The effect of convalescent plasma therapy on mortality among patients with COVID-19: systematic review and meta-analysis, Mayo Clin. Proc. 96 (5) (2021) 1262–1275.
[49] K.L. Kunze, P.W. Johnson, N. van Helmond, et al., Mortality in individuals treated with COVID-19 convalescent plasma varies with the geographic provenance of donors, Nat. Commun. 12 (1) (2021) 4864.
[50] C. Iannizzi, K.L. Chai, V. Piechotta, et al., Convalescent plasma for people with COVID-19: a living systematic review, Cochrane Database Syst. Rev. 5 (5) (2023) CD013600.
[51] F.K. Korley, V. Durkalski-Mauldin, S.D. Yeatts, et al., Early convalescent plasma for high-risk outpatients with covid-19, N. Engl. J. Med. 385 (21) (2021) 1951–1960.
[52] A. Alemany, P. Millat-Martinez, M. Corbacho-Monne, et al., High-titre methylene blue-treated convalescent plasma as an early treatment for outpatients with COVID-19: a randomised, placebo-controlled trial, Lancet Respir. Med. 10 (3) (2022) 278–288.
[53] B.A. Dickerman, H. Gerlovin, A.L. Madenci, et al., Comparative effectiveness of BNT162b2 and mRNA-1273 vaccines in U.S. Veterans, N. Engl. J. Med. 386 (2) (2022) 105–115.