Transfusing convalescent plasma as post-exposure prophylaxis against SARS-CoV-2 infection: a double-blinded, phase 2 randomized, controlled trial

Shmuel Shoham,1 Evan M Bloch,2 Arturo Casadevall,5 Daniel Hanley,3 Bryan Lau,6 Kelly Gebo,1 Edward Cachay,17 Seble G. Kassaye,11 James H. Paxton,24 Jonathan Gerber,15 Adam C Levine,10 Arash Naeim,16 Judith Currier,16 Bela Patel,13 Elizabeth S. Allen,18 Shweta Anjan,20 Lawrence Appel,1 Sheriza Baksh,6 Paul W. Blair,1 Anthony Bowen,1 Patrick Broderick,25 Christopher A Caputo,5 Valerie Cluzet,27 Marie Elena Cordisco,28 Daniel Cruser, Stephan Ehrhardt,6 Donald Fonthal,15 Yuiko Fukuta,9 Amy L. Gawad,3 Thomas Gniadek,13 Jean Hammel,26 Moises A. Huaman,14 Douglas A. Jabs,4 Anne Jedlicka,5 Nicky Karlen,7 Sabra Klein,5 Oliver Laeyendecker,29 Karen Lane,3 Nichol McBee,3 Barry Meisenberg,8 Christian Merlo,1 Giselle Mosnaim,12 Han-Sol Park,5 Andrew Pekosz,5 Joann Petrini,28 William Rausch,28 David M. Shade,6 Janna R. Shapiro,5 J. Robinson Singleton,22 Catherine Sutcliffe,6 David L. Thomas,1 Anusha Yarava,3 Martin Zand,21 Jonathan M. Zenilman,1 Aaron A.R. Tobian,2 David J. Sullivan5

1Department of Medicine, 2Department of Ophthalmology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA, 3Department of Pathology, and the 4Department of Ophthalmology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA, 5Department of Molecular Microbiology and Immunology and 6Department of Pathology, Northshor University Health System, Evanston, Illinois, USA, 7Department of Medicine, Division of Infectious Diseases, University of Cincinnati, Cincinnati, Ohio, USA, 15Department of Medicine, Division of Infectious Diseases, University of California, Irvine, Irvine, California, USA, 16Department of Medicine, Division of Infectious Diseases, University of California, Los Angeles, Los Angeles, California, USA, 17Department of Medicine, Division of Infectious Diseases and 18Department of Pathology, University of California, San Diego, San Diego, California, USA, 19Department of Medicine, Division of Hematology and Oncology, University of Massachusetts Chan Medical School, Worcester, Massachusetts, USA, 20Department of Medicine, Division of Infectious Diseases, University of Miami Miller School of Medicine, Miami, Florida, USA, 21Department of Medicine, University of Rochester, Rochester, New York, USA, 22Department of Neurology, University of Utah, Salt Lake City, Utah, USA, 23Department of Medicine, Division Critical Care Medicine, University of Texas Health, Houston, Texas, USA, 24Department of Emergency Medicine Wayne State University, Detroit, Michigan, USA, 25Danbury Hospital, 26Norwalk Hospital, 27Vassar Brothers Medical Center, Nuvance Health, Poughkeepsie, New York, USA and 28University of Vermont, Nuvance Health, Danbury, Connecticut, USA, 29Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Baltimore, Maryland, USA.

Corresponding author: Shmuel Shoham 1830 East Monument Street, Room 447, Baltimore, MD 21205; email sshoham1@jhmi.edu

© The Author(s) 2022. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com This article is published and distributed under the terms of the Oxford University Press, Standard Journals Publication Model (https://academic.oup.com/journals/pages/open_access/funder_policies/chorus/standard_publication_model)
ABSTRACT

Background. The efficacy of SARS-CoV-2 convalescent plasma (CCP) for preventing infection in exposed, uninfected individuals is unknown. CCP might prevent infection when administered before symptoms or laboratory evidence of infection.

Methods. This double-blinded, phase 2 randomized, controlled trial (RCT) compared the efficacy and safety of prophylactic high titer (≥1:320 by Euroimmun ELISA) CCP with standard plasma. Asymptomatic participants aged ≥18 years with close contact exposure to a person with confirmed COVID-19 in the previous 120 hours and negative SARS-CoV-2 test within 24 hours before transfusion were eligible. The primary outcome was new SARS-CoV-2 infection.

Results. 180 participants were enrolled; 87 were assigned to CCP and 93 to control plasma, and 170 transfused at 19 sites across the United States from June 2020 to March 2021. Two were excluded for screening SARS-CoV-2 RT-PCR positivity. Of the remaining 168 participants, 12/81 (14.8%) CCP and 13/87 (14.9%) control recipients developed SARS-CoV-2 infection; 6 (7.4%) CCP and 7 (8%) control recipients developed COVID-19 (infection with symptoms). There were no COVID-19-related hospitalizations in CCP and 2 in control recipients. Efficacy by restricted mean infection free time (RMIFT) by 28 days for all SARS-CoV-2 infections (25.3 vs. 25.2 days; p=0.49) and COVID-19 (26.3 vs. 25.9 days; p=0.35) was similar for both groups.

Conclusions. Administration of high-titer CCP as post-exposure prophylaxis, while appearing safe, did not prevent SARS-CoV-2 infection.

Keywords
SARS-CoV-2, post-exposure-prophylaxis, convalescent plasma, transfusion, COVID-19

Running title: SARS-CoV-2 exposure plasma prophylaxis or SARS-CoV-2 PEP with convalescent plasma
Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for Coronavirus Disease 2019 (COVID-19) and the pandemic that has claimed millions of lives [1]. Especially at the pandemic’s onset, effective preventive strategies were limited. Even by 2022, many have not been vaccinated, and some do not respond to vaccination [2, 3]. The urgency of effective prevention is highest within households of SARS-CoV-2 infected persons since 10-50% will be secondarily infected. Passive immunotherapy using preformed antibodies is effective as post-exposure prophylaxis (PEP) against many infections [4-7]. Combinations of monoclonal antibodies (mAb) are effective as COVID-19 PEP [8, 9]. COVID-19 convalescent plasma (CCP) may confer protection during early infection and in those without antibodies [10-12]. CCP has some advantages over mAb’s, including ease of procurement, low cost, and resilience against viral variants [13]. This study sought to evaluate the safety and efficacy of CCP containing anti-SARS-CoV-2 antibodies as PEP.

Methods

Study design and Participants

A randomized, double-blind, placebo-controlled clinical trial was conducted to compare the safety and efficacy of transfusion of CCP (intervention) with SARS-CoV-2 non-immune control plasma.

Asymptomatic participants aged ≥18 years who had a close contact exposure to a person with confirmed COVID-19 in the previous 120 hours and did not have SARS-CoV-2 vaccination, and past or active SARS-CoV-2 infection were eligible. The applied definition of close contact exposure was that used by Centers for Disease Control and Prevention (CDC) during the study period. Transfused participants positive by RT-PCR at screening were excluded from analyses. Participants were enrolled at 19 US centers between June 11, 2020 to June 23, 2021. Approval was obtained from the Institutional Review Boards at Johns Hopkins University School of Medicine functioning as single IRB for all participating sites. The protocol was additionally approved by the Department of Defense (DoD) Human Research Protection Office. An independent data and safety monitoring board provided oversight and reviewed efficacy and safety as the study was conducted. All participants provided written informed consent. Trial registration: Clinicaltrial.gov NCT04323800.

Randomization to treatment arm and masking

Eligible subjects were randomized 1:1 to receive a unit of CCP or control plasma using central interactive web-based systems. CCP and control plasma were in standard plasma bags, with identical labels.

Intervention

CCP donors were eligible for collection if they had a history of a positive molecular assay test result for SARS-CoV-2 infection, met standard criteria for blood donation, and had SARS-CoV-2 positive antibody levels after diluting 1:320 titer by Euroimmun ELISA, [Mountain Lakes, NJ] at screening. CCP was collected at various US locations. After qualification, the donor CCP antibody levels were later characterized in research laboratories by full length ancestral spike and receptor-binding domain (RBD) endpoint titers, live virus growth neutralization assays and Euroimmun arbitrary unit (AU) at the manufacturer’s recommended dilution of 1:101[14]. Control was standard SARS-CoV-2 non-immune plasma collected before January 1, 2020, or seronegative for SARS-CoV-2. Transfusions were performed at outpatient clinical research facilities. Individuals were followed for 90 days with visits at days 0 (transfusion), 1, 3, 7, 14, 28, 60, and 90. Nasal swabs were collected at screening (days -1 to 0) and at days 1, 7, 14, and 28. Assessments for COVID-19
(symptomatic infection) were conducted at screening, transfusion, and days 1, 3, 7, 14, 28, and 60. Safety assessments were continued to day 90. Viral testing was performed using RT-PCR that targeted the SARS-CoV-2 nucleocapsid gene. Recipient antibody levels were measured by RBD endpoint titers.

Primary Outcome

The primary efficacy outcome was incident SARS-CoV-2 infection by study day 28 by positive RT-PCR testing conducted on collected nasal swabs or by clinical RT-PCR testing conducted outside the study.

Secondary efficacy outcomes

Disease severity was measured to day 28 using a clinical event scale (supplementary material) and evaluated using an ordinal logistic model. Efficacy for preventing SARS-CoV-2 infection and COVID-19 was examined based on donor antibody titer through characterization of donor IgG, including end point titers and area under the curve (AUC) using a standardized ELISA to measure IgG against the spike and receptor binding proteins and anti-SARS-CoV-2 IgG against recombinant S1 domain of the SARS-CoV-2 spike protein (Euroimmun) as previously described [14].

Safety assessments

The Common Terminology Criteria for Adverse Events (CTCAE) 5.0 was used for grading of adverse events (AE). The safety outcomes were monitored throughout the study, including transfusion-related serious AEs (SAEs) (i.e. severe transfusion reactions, acute respiratory distress syndrome and grade 3 or 4 adverse events). The masked independent medical monitor evaluated AEs, SAEs and changes in baseline safety laboratory values.

Data management and statistical analyses

The pre-specified primary analysis of cumulative SARS-CoV-2 infection was conducted using a time-to-event analysis to compare the restricted mean survival time, referred to henceforth as restricted mean infection free time (RMIFT). We calculated and compared the restricted mean survival times by 28 days and risk difference (RD) by treatment arm in a modified intention to treat (mITT) analysis. We performed the primary analysis according to the participants’ original randomized treatment groups excluding those who did not receive a transfusion of study plasma and those who were later found to have been test positive at transfusion [15]. Analyses were adjusted for variables potentially related to the outcome in order to increase estimate precision (statistical analysis plan; supplementary material) [15]. Demographic and clinical variables were measured at baseline. To determine which pre-specified candidate variables to include, we conducted variable selection by random survival forest in the entire sample (i.e., not including an indicator term for treatment arm) and masked to treatment allocation. This algorithm was implemented on the mITT sample to identify the prognostic baseline variables for the entire sample.

Baseline characteristics are reported as proportions or medians with interquartile ranges (IQR) for continuous variables. Time-to-event analysis was computed from the time of transfusion until development of a positive molecular test for infection. Analyses were repeated using only clinical illness with COVID-19 as the outcome. Targeted minimum loss-based estimation (TMLE) was used for difference in RMIFT by 28 days and risk of infection. Time scale was days from transfusion. A one-sided test with type I error of 0.05 was used to determine statistical significance.

A secondary outcome was disease severity by day 28 using a clinical event scale ranging from no infection to death. The most severe status by the day 28 visit was ascertained using a TMLE estimator for ordinal outcomes and adjusted for the pre-specified candidate variables selected by the algorithmic approach [16, 17].
A pre-specified sensitivity analysis was restricted to participants who remained infection-free up to day 4 to account for people with early and still undetectable infection when CCP was administered and for an expected lag between transfusion and effect from passive antibody transfer. Since protocolized RT-PCR testing was not performed on days 2 and 3, it is possible that infected asymptomatic patients were included in this analysis.

*Donor antibody titers*

Analysis for donor spike antibody titer was conducted for AUC as a continuous variable: controls were assigned a value of zero. To model antibody effect, a flexible Weibull time to event model was used[18] to estimate the hazard ratios. To allow for non-linearity, both natural cubic splines and fractional polynomials were assessed choosing the model with the lowest Akaike Information Criterion (AIC) [19]. Data on days from donation to transfusion were collected and compared in the CCP vs placebo group.

*Safety*

Rates of severe transfusion reactions, AEs, grade 3 or 4 AEs, and death were evaluated by treatment arm; 95% confidence intervals (CI) were calculated using skewness-corrected asymptotic score for exact CI[20], using the R package 'ratesci'.

*Conditional Power Analysis*

The trial did not meet the target sample of 500 participants as enrollment stopped with widespread vaccine availability. The sample size calculation is provided as supplementary material. A conditional power analysis, using the R package ‘gsDesign’, was conducted to assess the likelihood of providing evidence for the efficacy of convalescent plasma.

*Results*

Of 1,138 participants screened, 180 (15·8%) were eligible and consented to the study and 170 were transfused (82 CCP; 88 control plasma; Figure 1). Of those transfused, two were excluded from efficacy analyses for baseline SARS-CoV-2 RT-PCR positivity. Table 1 lists participants’ demographic and baseline characteristics. Median time from exposure to transfusion was 2 days (IQR 1-4). Seven participants (3 CCP recipients) did not complete all study components.

CCP from 70 unique donations was transfused to 82 recipients; the IgG inverse endpoint titers to protein S were > 1,000 except for a single unit at 540. More than 85% of the plasma units were hospital qualified EUA high titer by Euroimmun Arbitrary units >3·5, international spike binding arbitrary units/mL > 60, RBD AUC>900 and virus 3-day culture neutralization > 8 International units/mL (appendix Figure S1).

*Primary Outcomes*

Of the 168 participants in the mITT analyses, 12/81 (14·8%) CCP and 13/87 (14·9%) control recipients tested positive for SARS-CoV-2 RNA. Three were positive on day 1 post-transfusion and 3 on days 2-3. The RMIFT by 28 days was 25·3 days for CCP and 25·2 for control recipients (p=0·47). The RD was 0·01 (p=0·42) lower for CCP. Excluding infections through day 3, the RMIFT was 26·6 days for CCP and 25·8 for control recipients (p=0·15). The RD was 0·04 (p=0·21) lower for CCP. Six (7·4%) CCP and 7 (8%) control recipients had COVID-19 (4 and 5 after day 3 from transfusion). The RMIFT by 28 days was 26·3 for the CCP and 25·9 days for the control recipients. The RD between groups was 0·012 lower for CCP. Excluding infections through day 3, the CCP group was consistently, but not significantly, better than control (difference in RMIFT =0·7 days, p=0·14; RD=0·017). Cumulative incidence of confirmed SARS-CoV-2 infections and of COVID-19, using a time-to-event analysis to compare the restricted mean survival (infection free) time are shown in figures 2 and 3.
Conditional power analyses were conducted since the target enrollment (500 transfused) was not reached. Had target enrollment been reached it is unlikely that statistically significant results would have been achieved, with chances for significant differences in RMIFT and RD calculated as 0·3% and 0·6% respectively.

**Adverse Events**

There were 86 reported AEs, of which 28 occurred with CCP and 58 with control plasma; 17/86 events were grade 3 or 4. Five participants required hospitalization (2 for COVID-19) all with control plasma (Supplemental Materials). CCP recipients had a lower proportion of any AEs (p=0·005), and severe AEs (p=0·06) (Table 2).

**Clinical Severity Score**

Two control participants required hospitalizations for COVID-19 (Table 3). The distribution of clinical severity was similar between the two groups for all events after transfusion (OR 0·99) and for events >3 days after transfusion (OR 0·94).

**Relationship between donor antibody levels and infection**

The donor antibody levels measured by binding to SARS-CoV-2 proteins or by virus neutralizations as well as the interval from plasma donation to transfusion was comparable in those infected or not infected when limiting analysis to those developing infection > 3 days after transfusion (Supplemental Figure 1 and 2). Pharmacokinetic analysis on 24 participants showed recipient antibody levels to be 4% or a 25-fold reduction from donor antibody levels with a 7-day recipient half-life measured over 14 days (Figure 4).

**Discussion**

This randomized, placebo-controlled, double-blinded trial evaluated the efficacy and safety of a unit of high antibody titer CCP for prevention of SARS-CoV-2 infection following recent, close contact exposure to a person with COVID-19. In this sample of outpatients, CCP did not reduce SARS-CoV-2 infection in participants transfused up to 120 hours following exposure.

The findings contrast with successful use of mAbs for PEP [8]. Inability of CCP to prevent infection cannot be ascribed to the absence of specific antibody to SARS-CoV-2, as both CCP and mAbs contain SARS-CoV-2 specific antibodies. Insufficient antibody dose in the CCP used is one explanation for lack of efficacy as PEP. The amount of immunoglobulin in the casirivimab/imdevimab dose is 12 grams, likely exceeding the amount of viral-specific antibodies in a unit of high-titer plasma. The concentration of antibodies in the casirivimab/imdevimab PEP trial was 22-25 mg/L which is about 150 times that needed for neutralization of many variants [21, 22]. CCP used in this study had geomean neutralizing international units/mL of 1:27, which when diluted about 30-fold after transfusion, resulted in 10-100 lower neutralizing capacity than mAbs. Neutralization potency of CCP may be impacted by multiple factors including time of plasma collection, distance from location of use, severity of illness and age. The impact of viral variants on efficacy of plasma collected earlier in the pandemic may be most profound with variants such as delta and omicron. Those would not have impacted the results of this trial. It is possible that low levels of the delta variant were present at the clinical sites during this trial, however, the treatment phase was completed prior to widespread circulation of those variants in the US. Qualitative differences between the products could also affect efficacy. For CCP, much of the neutralizing capacity is in IgM [23], a large molecule with poor tissue penetration; mAbs are entirely IgG, which has better tissue penetration[24].

Breakthrough SARS-CoV-2 infections despite vaccination provide insight as to why CCP did not prevent infection. Serum IgG is unlikely to prevent upper airways infection, presumably because of insufficient concentration within respiratory airway mucosa during initial infection when the epithelium is intact. As infection progresses an inflammatory response...
permits transudation of serum (and IgG) into tissues. Our results contrast with those of a study using the same plasma
supply, which found that CCP administered early in COVID-19 reduced hospitalization by 54% [25]. The large amount of
immunoglobulin in plasma after prophylactic mAb administration or vaccination is presumably sufficient to prevent
progression of infection to severe disease. However, the amount of specific antibody after a unit of CCP may be
insufficient to affect the course of initial infection, especially if much of the neutralizing antibody is IgM. This is
consistent with animal studies reporting antibodies’ inefficiency at reducing virus in nasal tissues [26]. Notably, two
control participants were hospitalized for COVID-19 (one with hematological disease and hypogammaglobulinemia).

Though the numbers are small, none of those who received CCP progressed to hospitalization, which is consistent with
findings that early treatment with passive immunotherapy (CCP or mAbs) reduces disease progression [10].

Our study affords insight into the optimal timing of CCP administration. Although numerous clinical trials and
observational studies were initiated early in the pandemic, these —overwhelmingly— focused on hospitalized patients
with severe COVID-19, collectively demonstrating little if any benefit in advanced disease [27]. By contrast, findings from
large observational studies of hospitalized patients suggested a mortality benefit when CCP was administered early [11].
Two clinical trials of CCP in outpatients (i.e., those with early infection) have also shown benefit. One showed a
significant reduction in progression of respiratory disease in older patients with COVID-19 who received CCP [10]. In the
US, the largest trial to date of outpatient CCP use demonstrated a 54% relative risk reduction in hospitalization relative
to controls [25]. Another trial enrolled patients with COVID-19 who presented to the emergency room (ER) [28]. It failed
to show a significant difference between the CCP and control arms, but the high number of patients (n=25) who were
hospitalized during the index visit, suggests that the trial may have selected for a population with more advanced
disease [28]. Our study, which focused on PEP refines our understanding of when CCP is optimally effective, thus
strengthening extant guidelines that recommend early use of qualified CCP following diagnosis [29].

Historically, convalescent serum was used for prophylaxis of measles [4] and mumps [5] where it was demonstrated to
prevent measles and mumps-related orchitis. These viruses are acquired by the respiratory route, but disease
manifestations are systemic [5]. For both measles and mumps, success of serum prophylaxis was measured by
prevention of systemic disease (rash and orchitis). These experiences suggest that it may be easier to prevent systemic
disease with antibodies than against respiratory tract-only disease. A similar pattern is found with pneumococcal
vaccine, in which antibodies are more effective in preventing sterile site than respiratory tract disease [30].

In this study, CCP was associated with substantially fewer Grade 3/4 and severe AE’s than control plasma. The reason for
this finding is unclear. As there were two hospitalization for COVID-19 in control recipients and none in CCP a possible
explanation could be protection from severe disease in those developing COVID-19 [25]. Early in the pandemic, there
were concerns about antibody-dependent enhancement (ADE) of infection [31, 32]. While ADE has not been reported in
CCP studies to date, almost all were conducted in hospitalized patients [33] and do not rule out the possibility of ADE in early infection when endogenous antibody responses are lacking. In this study, CCP was administered before or very
early in the course of infection and there was no evidence of ADE. This strongly suggests that ADE is not a significant
concern [31, 32, 34].

The study had limitations. The logistical challenges were formidable and frequently changed with the evolving pandemic.
Enrollment declined precipitously with widespread vaccine availability. Previously vaccinated individuals were ineligible
for participation, and guidance to defer vaccination until 90 days after receipt of CCP deterred potential subjects. The
enrollment goal of 500 total participants was not achieved. However, conditional power analyses for the primary
endpoint of infection suggest that results may not have significantly differed had the trial achieved target enrollment.
In conclusion, this RCT of high titer CCP given to participants exposed to, but not infected with SARS-CoV-2, within 120 hours demonstrated that CCP did not provide evidence of efficacy. Acknowledging the challenges of enrollment in the setting of vaccine availability, the ongoing evolution of SARS-CoV-2 with loss of multiple treatment and prevention options, could renew interest in new studies of CCP as PEP. Such studies should consider a higher dose of antibodies (i.e., collected from donors who have a history of SARS-CoV-2 infection and have also previously been vaccinated) and/or transfusion with multiple CCP units. Further, studies would best target populations most at risk, including the immunocompromised or elderly, with greater emphasis on clinical rather than laboratory outcomes.

NOTES

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online.

Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments.

The authors gratefully acknowledge the study participants who generously gave of their time and biological specimens. Initiation of this work was catalyzed by grants from Bloomberg Philanthropies and the State of Maryland

Financial Support.

This study was funded principally by the U.S. Department of Defense’s Joint Program Executive Office for Chemical, Biological, Radiological and Nuclear Defense (JPEO-CBRND), in collaboration with the Defense Health Agency (DHA) (contract number: W911QY2090012), with additional support from Bloomberg Philanthropies, State of Maryland, the National Institutes of Health (NIH) National Institute of Allergy and Infectious Diseases 3R01AI152078-01S1, NIH National Center for Advancing Translational Sciences U24TR001609-S3 and UL1TR003098, the Division of Intramural Research NIAID NIH, Mental Wellness Foundation, Moriah Fund, Octapharma, HealthNetwork Foundation and the Shear Family Foundation. The study sponsors did not contribute to the study design; the collection, analysis, and interpretation of data; manuscript preparation, and the decision to submit the paper for publication.

Potential conflicts of Interests.

The authors report KG- grants or contracts unrelated to this work and paid to institution from NIH; personal fees from Aspen Institute, Teach for America and UpToDate; TG- paid consultant for Fresenius Kabi USA and reports <$5,000 of JNJ stock; AC- Scientific Advisory Board of Sarotherapeutics (cow-derived human immunoglobulins COVID-19 treatment and other infectious diseases) and Ortho Diagnostics Speakers Bureau, and consulting fees from Ortho Diagnostics and Pfizer, and payment for expert testimony from King & Spalding LLP, and leadership or fiduciary role with American Society for Microbiology, and part owner of Melatech; EB- time is funded in part by National Heart Lung and Blood Institute (NHLBI) through grant 1K23HL151826, member of the FDA Blood Products Advisory Committee, Abbot Laboratories, Grifols Diagnostic Solutions, personal fee for invited educational presentations for Terumo BCT (honoraria for educational webinar) and advisor for California Institute for Regenerative Medicine (convalescent plasma program), and unpaid participation as invited member for a Data Safety Monitoring Board for the following trial: “Assessment of safety and efficacy of COVID-19 Convalescent Plasma for treatment of COVID-19 in adults in Uganda; A Phase III randomized controlled trial; SS research grants from Ansun, Astellas, Cidara, Emergent Biosolutions, F2G, Gilead, Merck, Scynexis, Zeteo, Shionogi and Shire, personal fees from Adagio, Adamis, Celltrion, Immunome, Intermountain Health and
Karyopharm (consultant, advisory board and data safety monitoring board member), participation on a Data Safety Monitoring Board or Advisory Board for Adagio, Adamis, Amplyx, Immunome, Intermountain Health, Janssen, Karyopharm, Reviral, and stock options from Immunome; DSu- grants or contracts unrelated to this work from NIH/NIAID (R01AI150763 Dual artemisinin action combats resistance; NIH R21TR001737 Quantum model repurposing of cethromycin for liver stage malaria; NIH R01AI111962 Optimized Combination Antimalarial Drug Therapy), founder, board member and stock options from AliquantumRx, DSMB member NIAID SMC/ISM Intramural 2018, medical royalties for malaria test (Binax Inc/D/B/A Inverness), consultant on malaria diagnosis for Masimo and Hemex Health and consulting fees for legal malaria case (Mabrey Firm 2019 and Ressler and Ressler 2018), and patents (Issued-USP 9,642,865 May 9, 2017 New angiogenesis inhibitors; Issued-USP 9,568,471 February 14, 2017 Malaria Diagnosis in Urine; Issued-USP 7,270,948 September 18, 2007 Detection of malaria parasites by laser desorption mass spectrometry; Pending SALTS AND POLYMORPHS OF CETHROMYCIN FOR THE TREATMENT OF DISEASE Patent Application (Application #20210163522); and Pending- Macrolide compounds and their use in liver stage malaria and related disease Application PCT/US2015/046665); EC- research grants from Gilead Sciences and Merck Sharp and Dohme (funds paid to UC Regents), payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Gilead Sciences, and advisory board member for Gilead Sciences; JC- consultant for Merck and Co. and Resverlogix; SKa- educational product development for Integritas Communications Group; GM- research grants from Teva, Sanofi Regeneron, Astra Zeneca, Alk Abello, Genentech, Propeller Health, GlaxoSmithKline and Novartis, and honoraria paid to author for HCPLive educational presentation, and position as Secretary/Treasurer (March 2019-February 2020), President-Elect (March 2020-February 2021), and President (March 2021-February 2022) of the American Academy of Allergy, Asthma and Immunology (payments made to institution during term as President) and Director of American Board of Allergy and Immunology (2020-2025), Co-Chair of American Board of Allergy and Immunology Continuous Assessment Program Examination Committee (2020-2022) (honoraria paid to author); CS- research grants from Centers for Disease Control and Prevention, Merck and Pfizer; DJ- grants or contracts unrelated to this work from National Eye Institute, National Institutes of Health and National Center for Advancing Translational Research, National Institutes of Health; Board of Directors of the American Uveitis Society, speaking honoraria from Retina Society, Controversies in Ophthalmology, University of Rochester, Wills Eye Hospital, LSU School of Medicine and Icahn School of Medicine at Mt. Sinai, and participation on a Data Safety Monitoring Board or Advisory Board for National Eye Institute Intramural Branch. DSh reports numerous grants supporting ongoing and completed research unrelated to this manuscript from the NIH; and is a member of DSMB for the Pelvic Floor Disorders Network (stipend support for meeting activities 4/year). DH reports consulting fees from Neurotrope. MH reports grants or contracts unrelated to this work from NIH National Center of Advancing Translational Sciences (NCATS) (KL2TR001426), NIH National Institute of Allergy and Infectious Diseases (NIAID) (UM1AI069501) and Insmed Inc, and is a member of the AIDS Clinical Trials Group (ACTG) Tuberculosis Transformative Science Group (TB TSG) Study Monitoring Committee. OL reports grants or contracts unrelated to this work from Division of Intramural Research, NIAID, NIH. VC reports stock(stock options from spouse’s employer and under spouse’s name from Pfizer. DT reports board membership with Excision Bio and board membership (DSMB) with Merck and Co (paid to author); employment with JHU; various expert testimony paid to author; honoraria for CME programs only, paid to author (no service on corporate speakers bureau); royalties from UpToDate; and stock(stock options with Excision Bio. NM reports participation as HyazOUT and UtahONE combined DSMB member for NIH National Center for Advancing Translational Sciences (NCATS) U24TR001609-S3. All other authors report no relevant disclosures.
References

1. WHO. WHO (COVID-19) Homepage. Available at: https://covid19.who.int/. Accessed June 12, 2021.

2. Ritchie H, Ortiz-Ospina E, Beltekian D, et al. Coronavirus (COVID-19) Vaccinations. Available at: https://ourworldindata.org/covid-vaccinations. Accessed June 13, 2021.

3. Boyarsky BJ, Werbel WA, Avery RK, et al. Antibody Response to 2-Dose SARS-CoV-2 mRNA Vaccine Series in Solid Organ Transplant Recipients. JAMA 2021; 325(21): 2204-6.

4. Gallagher JR. Use of Convalescent Measles Serum to Control Measles in a Preparatory School. Am J Public Health Nations Health 1935; 25(5): 595-8.

5. Rambar AC. MUMPS: Use of Convalescent Serum in the Treatment and Prophylaxis of Orchitis. American Journal of Diseases of Children 1946; 71(1): 1-13.

6. Luke TC, Casadevall A, Watowich SJ, Hoffman SL, Beigel JH, Burgess TH. Hark back: passive immunotherapy for influenza and other serious infections. Crit Care Med 2010; 38(4 Suppl): e66-73.

7. Hemming VG. Use of intravenous immunoglobulins for prophylaxis or treatment of infectious diseases. Clin Diag Lab Immunol 2001; 8(5): 859-63.

8. O’Brien MP, Forleo-Neto E, Musser BJ, et al. Subcutaneous REGEN-COV Antibody Combination to Prevent Covid-19. New England Journal of Medicine 2021; 385(13): 1184-95.

9. Cohen MS, Nirula A, Mulligan MJ, et al. Effect of Bamlanivimab vs Placebo on Incidence of COVID-19 Among Residents and Staff of Skilled Nursing and Assisted Living Facilities: A Randomized Clinical Trial. JAMA 2021; 326(1): 46-55.

10. Libster R, Pérez Marc G, Wappner D, et al. Early High-Titer Plasma Therapy to Prevent Severe Covid-19 in Older Adults. New England Journal of Medicine 2021.

11. Joyner MJ, Carter RE, Senefeld JW, et al. Convalescent Plasma Antibody Levels and the Risk of Death from Covid-19. N Engl J Med 2021; 384(11): 1015-27.

12. Thompson MA, Henderson JP, Shah PK, et al. Association of Convalescent Plasma Therapy With Survival in Patients With Hematologic Cancers and COVID-19. JAMA Oncology 2021; 7(8): 1167-75.

13. Casadevall A, Henderson JP, Joyner MJ, Pirofski LA. SARS-CoV-2 variants and convalescent plasma: reality, fallacies, and opportunities. J Clin Invest 2021; 131(7).

14. Klein SL, Pekosz A, Park HS, et al. Sex, age, and hospitalization drive antibody responses in a COVID-19 convalescent plasma donor population. J Clin Invest 2020; 130(11): 6141-50.

15. Diaz I, Colantuoni E, Hanley DF, Rosenblum M. Improved precision in the analysis of randomized trials with survival outcomes, without assuming proportional hazards. Lifetime Data Anal 2019; 25(3): 439-68.

16. Benkeser D, Diaz I, Luedtke A, Segal J, Scharfstein D, Rosenblum M. Improving precision and power in randomized trials for COVID-19 treatments using covariate adjustment, for binary, ordinal, and time-to-event outcomes. Biometrics 2020.

17. Diaz I, Colantuoni E, Rosenblum M. Enhanced precision in the analysis of randomized trials with ordinal outcomes. Biometrics 2016; 72(2): 422-31.
18. Royston P, Parmar MK. Flexible parametric proportional-hazards and proportional-odds models for censored survival data, with application to prognostic modelling and estimation of treatment effects. Stat Med 2002; 21(15): 2175-97.

19. Royston P. Model selection for univariable fractional polynomials. Stata J 2017; 17(3): 619-29.

20. Laud PJ. Equal-tailed confidence intervals for comparison of rates. Pharm Stat 2017; 16(5): 334-48.

21. Weinreich DM, Sivapalasingam S, Norton T, et al. REGEN-COV Antibody Combination and Outcomes in Outpatients with Covid-19. N Engl J Med 2021.

22. Copin R, Baum A, Wloga E, et al. The monoclonal antibody combination REGEN-COV protects against SARS-CoV-2 mutational escape in preclinical and human studies. Cell 2021; 184(15): 3949-61.e11.

23. Gasser R, Cloutier M, Prévost J, et al. Major role of IgM in the neutralizing activity of convalescent plasma against SARS-CoV-2. Cell Rep 2021; 34(9): 108790.

24. Burnett D. Immunoglobulins in the lung. Thorax 1986; 41(5): 337-44.

25. Sullivan DJ, Gebo KA, Shoham S, et al. Randomized Controlled Trial of Early Outpatient COVID-19 Treatment with High-Titer Convalescent Plasma. N Engl J Med. 2022 Mar 30. Epub ahead of print.

26. Zhou D, Chan JF, Zhou B, et al. Robust SARS-CoV-2 infection in nasal turbinates after treatment with systemic neutralizing antibodies. Cell Host Microbe 2021; 29(4): 551-63.e5.

27. Piechotta V, Iannizzi C, Chai KL, et al. Convalescent plasma or hyperimmune immunoglobulin for people with COVID-19: a living systematic review. Cochrane Database Syst Rev 2021; 5(5): Cd013600.

28. Korley FK, Durkalski-Mauldin V, Yeatts SD, et al. Early Convalescent Plasma for High-Risk Outpatients with Covid-19. New England Journal of Medicine 2021; 385(21): 1951-60.

29. Cohn CS, Estcourt L, Grossman BJ, et al. COVID-19 convalescent plasma: Interim recommendations from the AABB. Transfusion 2021; 61(4): 1313-23.

30. Webber C, Patton M, Patterson S, Schmoele-Thoma B, Huijts SM, Bonten MJ. Exploratory efficacy endpoints in the Community-Acquired Pneumonia Immunization Trial in Adults (CAPiTA). Vaccine 2017; 35(9): 1266-72.

31. Yager EJ. Antibody-dependent enhancement and COVID-19: Moving toward acquittal. Clin Immunol 2020; 217: 108496.

32. Dzik S. COVID-19 Convalescent Plasma: Now Is the Time for Better Science. Transfus Med Rev 2020; 34(3): 141-4.

33. Joyner MJ, Wright RS, Fairweather D, et al. Early safety indicators of COVID-19 convalescent plasma in 5000 patients. J Clin Invest 2020; 130(9): 4791-7.

34. Joyner MJ, Bruno KA, Klassen SA, et al. Safety Update: COVID-19 Convalescent Plasma in 20,000 Hospitalized Patients. Mayo Clin Proc 2020; 95(9): 1888-97.
### Table 1: Demographics and Medical Conditions at Randomization

|                                | Control Plasma (N=93) | Convalescent Plasma (N=87) |
|--------------------------------|-----------------------|---------------------------|
| Male, N (%)                    | 53 (57.0)             | 46 (52.9)                 |
| Race, N (%)                    |                       |                           |
| White                          | 78 (83.9)             | 80 (92.0)                 |
| Black                          | 6 (6.5)               | 4 (4.6)                   |
| Asian                          | 7 (7.5)               | 2 (2.3)                   |
| Native American                | 0 (0)                 | 0 (0)                     |
| Pacific Islander               | 0 (0)                 | 1 (1.1)                   |
| Other race                     | 2 (2.2)               | 0 (0)                     |
| Ethnicity, N (%)               |                       |                           |
| Hispanic/Latino                | 16 (17.2)             | 15 (17.2)                 |
| Age, median [min, max]         | 46.0 [18.0, 91.0]     | 48.0 [19.0, 82.0]         |
| Age category, N (%)            |                       |                           |
| 18-34                          | 26 (28.0)             | 18 (20.7)                 |
| 35-44                          | 18 (19.4)             | 19 (21.8)                 |
| 45-54                          | 19 (20.4)             | 22 (25.3)                 |
| 55-64                          | 16 (17.2)             | 14 (16.1)                 |
| ≥65                            | 14 (15.1)             | 14 (16.1)                 |
| BMI category, N (%)            |                       |                           |
| <18                            | 0 (0)                 | 2 (2.3)                   |
| ≥18-24.9                       | 34 (36.6)             | 23 (26.4)                 |
| ≥25-29.9                       | 14 (15.1)             | 30 (34.5)                 |
| ≥30-34.9                       | 16 (17.2)             | 10 (11.5)                 |
| ≥35-39.9                       | 11 (11.8)             | 6 (6.9)                   |
| ≥40                            | 5 (5.4)               | 3 (3.4)                   |
| Missing                        | 13 (14.0)             | 13 (14.9)                 |
| Number in household, N (%)     |                       |                           |
| 1                              | 26 (28.0)             | 18 (20.7)                 |
| 2                              | 21 (22.6)             | 19 (21.8)                 |
| 3                              | 15 (16.1)             | 17 (19.5)                 |
| 4                              | 10 (10.8)             | 17 (19.5)                 |
| >5                             | 17 (18.3)             | 12 (13.8)                 |
| missing                        | 4 (4.3)               | 4 (4.6)                   |
| Number of household positives, N (%) |                       |                           |
| 1                              | 54 (58.1)             | 54 (62.1)                 |
| 2                              | 5 (5.4)               | 8 (9.2)                   |
| 3                              | 3 (3.2)               | 1 (1.1)                   |
| ≥4                             | 1 (1.1)               | 0 (0)                     |
| Missing                        | 30 (32.3)             | 24 (27.6)                 |
| Median time from last exposure to transfusion (IQR) | 3 (1,4)               | 2 (1,4)                   |
| Days from last exposure to transfusion (170), N (%) |                       |                           |
| Cancer, N (%)                      | 7 (8.0) | 7 (8.5) |
|-----------------------------------|---------|---------|
| Active cancer                     | 1 (1.1) | 1 (1.1) |
| Active cancer on chemotherapy     | 1 (1.1) | 0 (0)   |
| Cancer in remission               | 5 (5.4) | 6 (6.8) |
| Leukemia/Lymphoma                 | 6 (6.5) | 2 (2.3) |
| **Cardiac Condition, N (%)**      |         |         |
| Arrhythmia                        | 1 (1.1) | 2 (2.3) |
| Atrial fibrillation, on anticoagulation | 0 (0) | 1 (1.1) |
| Cardiomyopathy                    | 0 (0)   | 1 (1.1) |
| Coronary artery disease           | 3 (3.2) | 1 (1.1) |
| Myocardial infarction             | 2 (2.2) | 0 (0)   |
| **Immunologic Condition, N (%)**  |         |         |
| Allergic rhinitis                 | 10 (10.8)| 12 (13.8)|
| Inflammatory bowel disease        | 3 (3.2) | 0 (0)   |
| HIV on antiretroviral treatment   | 6 (6.5) | 4 (4.6) |
| Psoriasis                         | 0 (0)   | 2 (2.3) |
| Immunosuppression on other immune modulator | 0 (0) | 1 (1.1) |
| **Metabolic Condition, N (%)**    |         |         |
| Diabetes mellitus                 | 5 (5.4) | 6 (6.8) |
| Vitamin D deficiency              | 1 (1.1) | 1 (1.1) |
| **Respiratory Conditions, N (%)** |         |         |
| Asthma                            | 5 (5.4) | 4 (4.6) |
| Chronic Bronchitis                | 2 (2.2) | 0 (0)   |
| Chronic sinusitis                 | 1 (1.1) | 0 (0)   |
| Cough                             | 1 (1.1) | 1 (1.1) |
| Pulmonary fibrosis                | 1 (1.1) | 0 (0)   |
| Pulmonary hypertension            | 1 (1.1) | 1 (1.1) |
| **Tobacco User, N (%)**           |         |         |
| Current tobacco user              | 1 (1.1) | 2 (2.3) |
| Past tobacco user                 | 4 (4.3) | 1 (1.1) |
Table 2: Adverse events

| Event                              | Control Plasma | Convalescent Plasma | Rate Difference (95% CI) | P-Value |
|------------------------------------|----------------|---------------------|--------------------------|---------|
| Incident Rate per 100 person-years (95% CI) | N   | N                 |                          |         |
| Severe transfusion reaction        | 1   | 0                 | -5 (-31, 19)             | 0.67    |
| Any adverse event                  | 58  | 28                | -147 (-254, -43)         | 0.005   |
| Grade 3 or 4 adverse event         | 13  | 4                 | -47 (-100, 2)            | 0.06    |
| Death                              | 0   | 0                 | 0 (-21, 23)              | 1       |

Table 3: Clinical severity in those receiving allocated intervention

| Event                                      | Control Plasma | Convalescent Plasma | Odds Ratio Model excluding events through day 3 (P-value) |
|--------------------------------------------|----------------|---------------------|----------------------------------------------------------|
| Incident Rate per 100 person-years (95% CI) | N=88 | N=82                |                                                          |
| Hospitalization for COVID-19               | 2   | 0                   |                                                          |
| No hospitalization, COVID-19              | 5   | 6                   | 0.99 (0.98)                                               |
| No hospitalization, asymptomatic SARS-CoV-2 infection | 6   | 6                   | 0.94 (0.90)                                               |
| No SARS-CoV-2 infection                    | 75  | 70                  |                                                          |
Figure Legends

Figure 1: Consort Diagram: Intention to treat analysis, including all transfused individuals. Those lost to follow-up between transfusion to end of follow-up contributed to the time at risk. Individuals with positive RT-PCR on day of transfusion were removed from analysis. * One randomized participant was found ineligible after randomization.

Figure 2: Cumulative incidence of confirmed infections and COVID-19.

Figure 3: Cumulative incidence confirmed SARS-CoV-2 infections and COVID-19 occurring after day 3.

Figure 4: The RBD AUC in the 24 donor plasma units were graphed at Day 0. Recipient participants (n=24) who were seronegative at screening and who did not acquire infection were measured for RBD protein antibody area under the curve (AUC) levels at day 1 (n=24), 7 (n=21) and 14 (n=20) after infection. Geometric means are shown. The recipient day 1 RBD levels were 4% of donor plasma levels. The half-life over days 1-14 is 7 days, half-life from day 1-7 is 4.5 days and over day 7 to 14 is 11.3 days.
Figure 1: CONSORT Diagram
(as of 23 June 2021)

Screening

Assessed for eligibility (n=2356)

Excluded (n=2176)
- Not meeting inclusion criteria at telephone screening (n=2145)
- Not meeting inclusion criteria (n=29)
- Declined to participate (n=2)

Randomized (n=180)

Allocation

Allocated to control plasma (n=93)
- Received allocated intervention (n=88)
- Did not receive allocated intervention (n=5)
  - Patient withdrew (n=4)
  - Unknown reason (n=1)

Allocated to convalescent plasma (n=87)
- Received allocated intervention (n=82)
- Did not receive allocated intervention (n=5)
  - Patient withdrew (n=4)
  - Adverse event during transfusion (n=1)

Follow-Up

Lost to follow-up (n=4)
- Loss to follow-up (n=4)
- Withdrawn from study (n=0)

Lost to follow-up (n=3)
- Loss to follow-up (n=3)
- Withdrawn from study (n=0)

Analysis

Analysed (n=87)
- Excluded from analysis (n=1)
  - Positive RT-PCR at transfusion (n=1)

Analysed (n=81)
- Excluded from analysis (n=1)
  - Positive RT-PCR at transfusion (n=1)
Figure 2
165x83 mm (.97 x DPI)

Figure 3
165x83 mm (.97 x DPI)
Figure 4