Hyperimmune Immunoglobulin for Hospitalized Patients With COVID-19:

A Randomized Controlled Trial

The ITAC (INSIGHT 013) Study Group

A complete list of members of the ITAC (INSIGHT 013) Study Group is provided in the Supplementary Appendix.

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Abstract

Background

Passive immunotherapy using hyperimmune intravenous immunoglobulin (hIVIG) to SARS-CoV-2, derived from recovered donors, is a potential rapidly available, specific therapy for an outbreak infection such as SARS-CoV-2. Findings from randomized clinical trials of hIVIG for the treatment of COVID-19 are limited.

Methods

In this international randomized, double-blind, placebo-controlled trial (NCT04546581), hospitalized patients with COVID-19 who had been symptomatic for up to 12 days and did not have acute end-organ failure received a single infusion of hIVIG, made by one of 4 manufacturers, or placebo, in addition to remdesivir, when not contraindicated, and other standard clinical care. Follow-up was for 28 days. The primary outcome, pooled over the 4 hIVIG manufacturers, was measured at day 7 by a seven-category ordinal endpoint that considered pulmonary status and extrapulmonary complications and ranged from no limiting symptoms to death. The primary endpoint is summarized with an odds ratio (OR) from a proportional odds model adjusted for the patient's ordinal category at entry and the manufacturer who provided the hIVIG product. ORs > 1.0 favor the hIVIG group. Deaths and adverse events, including organ failure and serious infections, were collected and used to define composite safety outcomes at days 7 and 28; ORs and hazard ratios (HRs) are cited and for these outcomes estimates < 1.0 favor the hIVIG. Pre-specified subgroup analyses were carried out for efficacy and safety outcomes by duration of symptoms, the presence of anti-spike neutralizing antibodies and other baseline factors.

Results

593 participants were enrolled, 579 included in the modified intention to treat analysis. Compared with placebo, the hIVIG arm did not have significantly greater odds of a more favorable outcome at day 7; the

adjusted odds ratio (OR) was 1.06 (95% CI 0.77 – 1.45, p=0.72). Infusions were well-tolerated, though infusion reactions were more common in the hIVIG arm (18.6% versus 9.5% for placebo, p=0.002). The percentage with the composite safety outcome at day 7 was similar for the hIVIG (24%) and placebo groups (25%)(OR=0.98; 95% CI: 0.66 – 1.46; p=0.91). The ORs for the day 7 ordinal outcome did not vary for subgroups considered, but there was evidence of heterogeneity of the treatment effect for the day 7 composite safety outcome: risk was greater for hIVIG compared to placebo for those antibody positive, OR (hIVIG/placebo) = 2.21 (95% CI: 1.14-4.29), while for those antibody negative, the OR was 0.51 (95% CI: 0.29-0.90) (p=0.001 for interaction).

Conclusion

When administered with standard of care including remdesivir, SARS-CoV-2 hIVIG did not demonstrate efficacy among patients hospitalized with COVID-19 without end-organ failure. The safety of hIVIG may vary by the presence of endogenous neutralizing antibodies at entry.

Research in Context

Evidence before this study

Passive immunotherapies targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been considered promising potential therapies for coronavirus disease 2019 (COVID-19) since the beginning of the pandemic. Convalescent plasma (CP) has been in wide use particularly early in the epidemic, while more recently monoclonal antibodies directed at SARS-CoV-2 and polyclonal hyperimmune immunoglobulin (hIVIG) to SARS-CoV-2 derived from recovered donors have emerged as potential therapies.

We searched PubMed for research articles published between database inception and December 15, 2021, for clinical trials of anti-SARS-CoV-2 passive immunotherapies among hospitalized patients with COVID-19 using various combinations of the terms “COVID-19,” “SARS-CoV-2,” “monoclonal antibody” “convalescent plasma” “intravenous immunoglobulin” “passive immunotherapy” and “clinical trial.” No language or date restrictions were applied. One small parallel-group trial reported encouraging results for treatment with hIVIG among hospitalized patients with COVID-19. Two trials (bamlanivimab in a trial conducted by the ACTIV-3 investigators, and casirivimab/imdevimab in a trial conducted by the RECOVERY investigators) reported no clinical benefit for anti-SARS monoclonal antibody therapy for the broad population of hospitalized patients with COVID-19 but suggested potential benefit for patients without endogenous anti-SARS-CoV-2 antibodies at the time of treatment. Trials for CP varied greatly in their size, population and rigor; taken together these trials including the largest (conducted by the RECOVERY investigators) showed no clinical benefit of CP in the hospitalized population.

Added value of this study

This study is the first well-powered, controlled clinical trial to report results of hIVIG for the treatment of hospitalized patients with COVID-19. When administered with standard of care including remdesivir,

SARS-CoV-2 hIVIG did not demonstrate efficacy among patients hospitalized with COVID-19 without end-organ failure. There was no heterogeneity of treatment effect in efficacy among patients without compared to those with endogenous antibodies, but there was heterogeneity of treatment effect for the primary safety outcome: risk was greater for hIVIG compared with placebo for those with endogenous neutralizing anti-SARS-CoV-2 antibodies at the time of treatment.

Implications of all the available evidence

Clinical trials completed to date do not support use of antibody-based passive immunotherapies including CP, mAbs, and hIVIG for the broad population of hospitalized patients with severe COVID-19. Unlike some trials of mAbs, this trial did not show evidence of benefit in those without endogenous neutralizing anti-SARS-CoV-2 antibodies at the time of treatment, but did suggest that safety of hIVIG and potentially other passive immunotherapies may vary by baseline antibody status. Further evaluation could better define the appropriate target population for this and other passive immunotherapies against SARS-CoV-2.

Introduction

Current effective therapies for individuals hospitalized with coronavirus disease 2019 (COVID-19) target viral replication or pathological elements of the host inflammatory response[1-4]. However, morbidity and mortality persist and additional treatments are urgently needed.

Augmenting the host humoral immune response to SARS-CoV-2 via passive immunotherapy is one possible therapeutic approach. Development of endogenous neutralizing antibody responses to SARS-CoV-2 appears variable and may not be present by the time of hospitalization[5-7].

Approaches using engineered monoclonal antibodies (mAbs) targeting viral elements have shown benefit among outpatients early in the course of COVID-19[8-9] For hospitalized patients, results from two trials of mAbs indicate that the clinical benefit and possibly safety of mAbs for hospitalized patients may depend on the presence of endogenous neutralizing antibodies (nAbs) at the time of randomization[10-12].

Convalescent plasma (CP) from recovered donors has been studied in both non-randomized and randomized trials for a variety of infectious diseases. With few exceptions[13,14], randomized trials have not shown consistent evidence of benefit. In COVID-19, one small study in older outpatients early in the course of COVID-19 showed benefit[14], but this result has not been consistently replicated[15]. In hospitalized patients, while a non-randomized study found that risk of death was reduced for hospitalized patients given CP that had higher anti-SARS-CoV-2 IgG antibody levels compared to those given CP with lower antibody levels[16], overall randomized trials have not consistently shown that CP reduces the risk of death or improves outcomes[16-21]. Reasons for this may include variability in the titer of specific antibodies in convalescent plasma.

Hyperimmune intravenous immunoglobulin (hIVIG)is derived from healthy individuals who have recovered from COVID-19 and mounted a neutralizing immune response to the infection[22]. It differs from CP in being a drug product manufactured from plasma pooled from multiple donors. It is

comprised of purified immunoglobulin G in a limited volume, and is standardized to high neutralizing titers to SARS-CoV-2, thereby overcoming the inter-unit variability of CP. Unlike mAbs, hIVIG is a concentrated mixture of polyclonal antibodies reflecting the diversity of the endogenous antibody response, which may provide advantages over mAbs by mitigating immune escape by viral variants. A small single-centre trial reported encouraging results with this approach[23]; parallel approaches using polyclonal product derived from non-human inoculation strategies also suggested possible benefit[-24] Findings from well-powered, controlled clinical trials of hIVIG to SARS-CoV-2 have not been reported.

We conducted a randomized, double-blind, placebo-controlled trial to evaluate the safety and clinical efficacy of anti-SARS-CoV-2 hIVIG in addition to standard of care including the antiviral remdesivir in individuals hospitalized with COVID-19 without end-organ failure between October 2020 and March 2021. Following the completion of the trial stored specimens collected at study entry were analyzed to address an *a priori* hypothesis that patients without nAbs at study entry would benefit more from hIVIG compared to placebo that those with nAbs.

Methods

This was an international, double-blind, placebo-controlled trial conducted by the International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) at sites in the United States, Europe, Africa, Asia, Latin America and the Middle East.

The protocol and statistical analysis plan are available in supplementary materials. Methods are summarized below.

Population

Participants were adults (≥18 years) hospitalized with documented SARS-CoV-2 infection and symptoms attributable to COVID-19 for 12 days or less. Exclusion criteria included prior passive immunotherapies, end-organ failure (including vasopressor therapy, new renal replacement therapy, and mechanical ventilation), known IgA deficiency with anti-IgA antibodies, and certain thrombotic conditions and pro-thrombotic disorders. Additional eligibility criteria are provided in the Supplementary Appendix.

The protocol was approved by a central institutional review board or an ethics committee at each participating site. Written informed consent was obtained from all participants or their legally authorized representative. The protocol is registered at ClinicalTrials.gov (NCT04546581).

Hyperimmune Intravenous Immunoglobulin (hIVIG) Products

A dose of hIVIG of 400mg per kilogram body weight, capped at 40g, was chosen based on predicted efficacy from in vitro studies of SARS-CoV-2 neutralization activity, prior safety data for non-COVID hIVIG products, and consideration of likely tolerability of the required infusion volume in this patient population. Given the early scarcity of SARS-CoV-2 hIVIG, four products were used: CSL Behring (King of Prussia, PA), Emergent BioSolutions (Gaithersburg, MD), Grifols S.A (Barcelona, Spain) and Takeda (Osaka, Japan) each manufactured hIVIG for the study using plasma collected either from fractionated whole blood or by plasmapheresis from healthy convalescent volunteers at sites in North America and

Europe. Donors and plasma units were selected on the basis of neutralization antibody titres against SARS-CoV-2. All hIVIG lots underwent central testing and were required to meet a prespecified range of neutralizing activity (potency) (Supplementary Appendix).

Infusion of hIVIG/placebo was to commence at a rate of 0.5/mg/kg/minute for approximately 30 minutes. If tolerated, the rate of infusion could be doubled after intervals of not less than 30 minutes up to a maximum of 4 mg/kg/minute.

Each site pharmacy was allocated the same hIVIG product throughout the trial; a single site pharmacy could serve multiple sites. Data from participants receiving each of the products and corresponding placebo were pooled for the primary analysis. Each participant receiving hIVIG received product from a single lot.

Standard Care

All participants received supportive care reflecting local practice and national guidelines. Standard of care background therapy included up to ten days of study-provided remdesivir unless contraindicated. Other aspects of standard care including corticosteroids, prophylactic anti-coagulation, supplemental oxygen, and other end-organ support were administered as clinically indicated.

Randomization and Blinding

Participants were randomly assigned (1:1) to receive either hIVIG or an equivalent volume of saline as placebo. Randomization was stratified by site pharmacy; schedules were prepared using a mass weight urn design[25]. Infusions were prepared by trial pharmacists and masked using opaque sleeves. All other investigators and research staff, and trial participants were blinded to the treatment administered.

Laboratory Measurements

SARS-CoV-2 viral RNA levels were measured from a mid-turbinate nasal swab. Plasma samples collected at study entry were used to measure anti-spike receptor binding domain (RBD) neutralizing antibodies (nAbs), and anti-nucleocapsid (anti-N) binding antibody levels. Plasma SARS-CoV-2 N antigen was measured using a microbead-based immunoassay. These centrally determined measurements were used to address pre-specified subgroup hypotheses (see supplemental appendix).

Outcome Measures

The primary endpoint was an ordinal outcome based on the patient's clinical status on Day 7 (Figure 1 and Supplementary Appendix). The seven categories of this outcome ranged from return to usual activities with no more than minimal symptoms due to COVID-19, to death. They reflect oxygen requirements and a range of organ dysfunction and were modified from similar outcomes in prior influenza and COVID-19 studies[1,12,26-27]. The primary safety outcome was a composite of death, serious adverse events (SAEs) and grade 3 or 4 adverse events through day 7. SAEs included organ failure events and serious infections which were reported as secondary endpoints separately from other serious adverse events (see supplemental appendix). Adverse events were graded for severity using the toxicity table of the Division of AIDS, NIAID, version 2.1. Adverse events were categorized according to codes in the Medical Dictionary for Regulatory Activities (MedDRA®), Version 23.1. The composite safety outcome at day 28 included all of the outcomes used in the day 7 safety outcome except grade 3 and 4 adverse events. Several other outcomes were specified in the protocol or statistical analysis plan; all protocol-defined outcomes are summarized in the supplemental appendix

Participants were followed for 28 days from randomization.

Statistical Analysis

The planned sample size was 500 participants (250 per arm). This sample size provides 80% power to detect an OR (hIVIG versus placebo) of 1.61 for a more favorable outcome at day 7 on the ordinal scale at the 0.05 (2-sided) level of significance (see supplemental appendix).

Analyses were performed on a modified intention to treat (mITT) population, which included all randomized participants who met eligibility criteria and received all or part of the assigned study product infusion. A proportional odds model was used to compare the primary ordinal outcome at day 7. The proportional odds model estimates a summary odds ratio (OR); that is, the ratio of the cumulative odds of being in a better category of the ordinal outcome for hIVIG versus placebo. ORs greater than 1.0 correspond to more favorable outcomes for those receiving hIVIG. Models were adjusted for pulmonary status at entry and which of the four hIVIG products was provided to the site. The day 7 primary outcome was imputed for participants for whom this information was missing (see supplemental appendix).

A logistic regression model was used to estimate an OR for hIVIG versus placebo for the composite safety endpoint through day 7. Percentages of participants who experienced infusion reactions or prematurely terminated infusions were also compared between treatment arms using logistic regression. Each of these logistic regression models adjusted for baseline ordinal outcome category and hIVIG study product provided to the site. The composite safety outcome at day 28 was summarized with hazard ratios (HRs) estimated from a proportional hazards model adjusted for baseline ordinal outcome category and hIVIG product provided to the site. ORs and HRs < 1.0 for these safety outcomes favor hIVIG compared to placebo. We tested the proportional hazards assumption by including an interaction term between the treatment indicator and log-transformed follow-up time.

Kaplan-Meier estimates were used to summarize time to the day 28 composite safety outcome, time to the three least favorable categories of the ordinal outcome, and time to discharge or the most favorable category on the ordinal scale. For the latter outcome, Gray's test with rho=0, the Fine-Gray model for stratified models, and the Aalen-Johansen estimator for the cumulative incidence curve which is the competing risk equivalent to the Kaplan-Meier estimator and Cox proportional hazards models are used[28-30]. A recovery rate ratio (RRR) is cited for this outcome, also adjusted for baseline ordinal outcome category and hIVIG product provided to the site; estimates > 1.0 denote superiority of hIVIG to placebo.

Subgroup analyses were carried out for the primary ordinal outcome at day 7 and the composite safety outcome at day 7. Heterogeneity was assessed by including interaction terms in the proportional odds and logistic regression models. Key *a priori* defined subgroups are described in the supplemental appendix and statistical analysis plan.

Statistical analyses were performed with the SAS software, version 9.4. All p values reported are 2-sided.

Funding Statement

The study was funded by the U.S. National Institutes of Health. Except for named members of the writing and study group, the funder had no role in data collection, analysis, interpretation, writing of the manuscript or the decision to submit.

Results

Participant Characteristics

From October 8, 2020 to February 10, 2021, 593 participants (301 hIVIG, 292 placebo) were enrolled at 63 sites in 11 countries (Tables S1 and S2); 295 participants in the hIVIG arm and 284 in the placebo arm (579 total) were in mITT analysis cohort (Figure S1). The number of participants given each of the four products or its matching placebo and potency levels for lots of the products were similar (Tables S3 and S4).

Demographic and clinical characteristics were similar between groups, with exception of sex where women comprised 49% of the hIVIG group and 37% of the placebo group (Table 1).

The median time from onset of first COVID-19 symptoms to participant randomization was 8 days (IQR 6-10); 38% were receiving either supplemental oxygen ≥ 4 liters per minute or high-flow oxygen. 96% of participants received remdesivir; 49% had started remdesivir prior to randomization. 56% were receiving corticosteroids and 61% received at least a prophylactic dose of heparin prior to randomization (Table 1 and Table S5).

Baseline endogenous anti-SARS-CoV-2 antibody and antigen levels were completed for 539 (93%) patients; 261 (48%) were positive for anti-Spike nAbs and 374 (69%) were positive for anti-Nucleocapsid Abs; 507 (94%) had detectable plasma nucleocapsid antigen levels. The median (IQR) for antigen was 1,368 (206, 4,335) ng/L. Viral RNA was detected in the central reference lab among 438 of 513 with mid-turbinate swab material; median (IQR) RNA viral load among those RNA positive was 169,979 (7,261, 2,147,457) copies/mL.

The presence of endogenous nAbs varied by duration of symptoms at entry, ranging from 27% for those with symptoms < 6 days to 67% for those with symptoms 10-12 days (Table S6).

All but 2 patients were infused with hIVIG/placebo on the day of randomization. Randomization occurred within 2 days of admission for 81% of patients.

Efficacy Outcomes

Primary Efficacy Outcome

The primary ordinal outcome at day 7 was available for all but 7 participants (Figure S1). Outcomes were imputed for these 7 participants (Supplementary Appendix). The OR for being in a more favourable outcome in the hIVIG arm compared to placebo on day 7 was 1.06 (95% CI 0.77 – 1.45) p=0.72 (Figure 1 and Table 2). The proportional odds assumption was met (p=0.97). Planned sensitivity analyses for the primary endpoint analysis given in Table S7 yielded consistent results.

The summary ORs for the ordinal outcome on days 3, 5, 14 and 28 ranged from 0.96 to 1.09 (Table S8). When comparing the day 7 ordinal category with baseline ordinal category, 63% of participants in the hIVIG arm and 64% in the placebo arm were in a better category; 15% and 18% respectively were in a worse category (Figure 1 and Table S9).

Other Efficacy Outcomes

Treatment differences were not significant for any of the other efficacy outcomes (Table 2, Tables S10, S11, S12, and S13). The RRR for time to discharge or the most favourable category of the primary ordinal outcome was 1.07 (0.92-1.26); p=0.37 (Figure 1C and Table 2).

Safety Outcomes

Infusion reactions were significantly more common in the hIVIG arm: for reactions of any grade, 19% of participants in the hIVIG group compared with 10% for placebo (p=0.002) (Table S14); and for reactions grade 3 or higher 6% of subjects in the hIVIG arm compared with 1% for placebo (p=0.012) (Table 2). Infusions were paused for an adverse event in 7% of participants in the hIVIG group and 3% in the

placebo group (p=0.01) (Tables S15). These differences between treatment groups remained significant after the exclusion of one site which infused at a faster rate for all of their participants (Tables S17-S19).

In the hIVIG group, 24% of participants experienced the composite safety outcome through day 7 compared to 25% in the placebo group (OR 0.98; 95% CI: 0.66 – 1.46); p=0.91) (Table 2).

Components of the composite safety outcome through day 7 are summarized in supplemental Tables S20, S21 and S22. Grade 3 or 4 adverse events and organ failure or serious infection outcomes were the most common occurring components of the composite safety outcome through day 7. The most commonly reported safety outcomes were respiratory (Tables S21 and S22). Respiratory events, including respiratory failure, defined as an increase in oxygen requirements to high flow nasal cannula, non-invasive ventilation, or mechanical ventilation, and grade 3 or 4 adverse events corresponding to MEDdra Preferred Terms of dyspnea, hypoxia, and respiratory failure, occurred in 14% of hIVIG patients and 18% of placebo participants (OR=0.72; 95% CI: 0.45 – 1.16; p=0.18). The HR for the day 28 composite safety outcome was 0.79 (95% CI: 0.57-1.11) (Tables 2 and S20, Figure S2). There was no evidence that the proportional hazards assumption was violated (p=0.33). Similar to the events occurring through day 7, most events through day 28 were due to respiratory failure (Tables S23 and S24).

Through day 28, 18 deaths (6%) occurred in the hIVIG group and 22 deaths (8%) occurred in the placebo group, HR 0.80 (0.42 – 1.51); p=0.49 (Table 2).

Adverse events of any grade severity at days 1, 3, 7 and 28 are summarized in Tables S25-S28.

Changes in laboratory safety parameters and in concomitant medications are summarized in Tables S29, S30 and S31.

Subgroup Analyses

Subgroup analyses for the primary ordinal outcome at day 7 and the composite safety outcome at day 7 are summarized in Figures 2 and 3, respectively, and the supplemental appendix (Table S32 and S33). For the primary ordinal outcome at day 7 there was no evidence of treatment effect modification for any of the subgroups considered. As expected, the ORs for each of the hIVIG products did not vary (p=0.95 for interaction). Contrary to our hypothesis, when considering days from symptom onset to randomization, the OR was less than 1.0 (OR=0.74), favoring placebo, for those with symptom onset < 6 days (the category for which the most favourable treatment effect was expected). ORs exceeded 1.0, favoring hIVIG, for those with later symptom onset (categorised as 6-7, 8-9, and 10-12 days). There was also no evidence for a different treatment effect for those nAb negative (OR=0.97) and positive (OR=1.02) (p=0.79 for interaction) at baseline. Significant treatment effect heterogeneity was also not found for subgroups defined by the presence of anti-N Abs, antigen level, viral RNA level, and the combination of nAb levels and antigen level and viral RNA levels (Table S32).

In contrast, for the composite safety outcome through day 7, a significant interaction was evident by nAb status (Figure 3 and Table S33). Among those nAb positive at baseline, 26.3% of patients in hIVIG group and 16.4% in the placebo group experienced at least one event included in the composite safety outcome (OR= 2.21; 95% CI: 1.14-4.29). For those nAb negative at baseline 22.7% of patients in hIVIG group and 34.3% in the placebo group experienced at least one event included in the composite safety outcome (OR=0.51; 95% CI: 0.29-0.90) (p=0.001 for interaction). The increased risk of the composite safety outcome among those nAb positive was evident for those with high and low antigen/viral RNA levels (Table S33).

At day 28, the HRs for composite safety outcome did not differ for the subgroups defined by nAb status (p=0.18 for interaction) (Table S34 and Figures S3 and S4). The day 7 increased risk of the composite safety outcome for nAB positive participants was no longer evident at day 28 (HR=1.01; 95% CI: 0.57-1.79); the cumulative percentages with a composite safety outcome at day 7 were 15.8% and 12.5% for

the hIVIG and placebo groups and at day 28 were 18.9% and 20.9% for the hIVIG and placebo groups, respectively (Figure S3). In contrast, for those nAb negative at baseline, the day 7 reduced risk of the composite safety outcome persisted through day 28 (HR=0.62; 95% CI: 0.39-0.98); by day 7 the cumulative percentages with a composite safety outcome were 14.9% and 25.5% for the hIVIG and placebo groups, respectively, and 24.9% and 35.9% for the two groups by day 28 (Figure S4). As is evident from these percentages and comparing the curves on Figures S3 and S4, risk of the composite safety outcome was greater for those nAb negative than those nAb positive in both treatment groups.

The components of the day 7 and day 28 composite safety outcomes by nAb category are summarized in Tables S35-S44. Through both day 7 and day 28, end organ disease events, specifically respiratory failure, were the most common events for those nAb positive and nAb negative.

Discussion

In this randomized double-blind, placebo-controlled trial among hospitalized patients with COVID-19 with up to 12 days of symptoms and no end-organ failure, there was no evidence that those who received a single infusion of hIVIG in addition to remdesivir and other standard of care had better clinical outcomes at day 7 following randomization than those who received placebo plus remdesivir and standard of care. This finding was mirrored in secondary efficacy outcomes, with no differences observed in clinical status at other time-points, or in time to discharge or to the most favorable category of ordinal outcome through day 28. Overall, these findings indicate that hIVIG confers no clinical benefit for hospitalized patients with COVID-19.

Infusion reactions were more common in those receiving hIVIG compared to placebo but most were of low-grade severity. The percentage experiencing the composite safety outcome (including deaths, SAEs, end organ disease and serious infections, and grade 3 and 4 events) through day 7 did not differ between the treatment arms.

The failure to observe efficacy of hIVIG in this study could be explained in a number of ways. A key possibility is that antibody therapy may not benefit those who have already mounted an immune response. Thus, the null result overall could reflect the balance of a positive response in the antibody negative subgroup and a neutral or unfavorable response in the antibody positive subgroup. In addition, it is possible that other characteristics of progressive COVID-19 affect the utility of hIVIG: systemically infused antibody may not effectively penetrate lung tissue in the pneumonic phase of the illness, while some patients may have progressed the inflammatory phase of COVID-19 in which augmenting the humoral immune response may not useful[31]. It is also possible that the antiviral effects of hIVIG beyond those of remdesivir are insufficient to be detected.

We hypothesized that there may be a critical time-dependency of the impact of antibody therapy in patients with COVID-19. *A priori* defined subgroup analyses were defined to address this. Contrary to our pre-specified hypotheses there was no evidence of benefit based on the day 7 ordinal outcome in those treated earliest or in patients without endogenous nAbs at entry. Among those treated within 6 days of symptom onset (23% of participants) the odds of a favorable outcome with hIVIG was, in fact, lower than placebo, giving a relative odds of 0.74. Among the 48% of patient who were nAb negative the relative odds (hIVIG/placebo) of a favorable outcome was 0.99 and not different from those who were nAb positive at study entry. There was no difference in treatment effect by baseline measures of systemic inflammation: subgroup analyses by C-reactive protein at entry did not reveal a differential treatment effect for either the day 7 ordinal outcome or the composite safety outcomes assessed at day 7 and day 28.

Comparison of these subgroup findings with two recent studies of mAbs in similar hospitalized populations demonstrates significant differences. In the RECOVERY study of combination casirivimab and indevimab there was no benefit seen in overall mortality for the general hospitalized population; however an analysis population of those negative for SARS-CoV-2 binding antibodies at baseline showed a significant mortality reduction in the mAb arm[11]. Similarly, the ACTIV-3/TICO study of bamlanivimab showed no overall benefit in a hospitalized population, but an improvement in time to sustained recovery in those nAb negative at entry[12].

Evaluation of any impact of differences in the viral variants and the antibody responses to those variants in the hIVIG also requires consideration in understanding the overall lack of clinical benefit. Plasma for IVIG was collected in North America and Europe during the summer of 2020 and the trial enrolled across those and other regions during the winter of 2020/2021. However, enrolment was largely complete prior to widespread emergence in enrolling countries of SARS-CoV-2 variants with potential immune escape characteristics such as B.1.1.7 (alpha), B.1.351 (beta)[3].

It is possible that the infusion of hIVIG led to harm in some patients. While there was no overall difference in the composite safety outcome at day 7 or day 28, among those nAb positive at entry an increased risk of safety events was observed, giving a relative odds for a safety event of 2.21 at day 7, although no difference was seen when considering safety events through day 28. At both day 7 and day 28 for nAb negative participants the risk of the composite safety outcome was lower in the hIVIG compared to the placebo group. The majority of safety events were of a respiratory nature in both the nAb positive and nAb negative groups, including increasing dyspnea, increasing oxygen requirements, and respiratory failure. The treatment effect for a similar outcome also varied according nAb status in the placebo-controlled trial of the mAb bamlanivimab in hospitalized patients[12]. Taken together, the findings of this trial of hIVIG and of other trials of passive immunotherapies suggest that such therapies may be associated with harm in some hospitalized patients and benefit in others.

Elucidating the mechanisms of any possible harm of hIVIG in nAb positive individuals will require further study. One study of CP in hospitalized patients suggested certain antibody compositions, specifically the presence of IgG against the full transmembrane spike protein, could be associated with adverse events[19]. Pre-existing antibodies to type I interferons have been associated with risk of COVID progression and it is also possible that passive transfer of these antibodies could have adverse effects[33], though any impact would be expected to be mitigated in the pooled hIVIG product. Other theoretical possibilities include development of antibody-dependent enhancement with exaggerated viral infectivity and inflammation[34-35], formation of antibody complexes in those with pre-existing neutralizing antibodies to SARS-CoV-2, or adverse inflammatory effects via Fc-mediated antibody functions[36].

These findings have certain limitations. While the sample size was sufficient to exclude a OR in favour of hIVIG of 1.61 with 95% confidence, the sample size may not have allowed detection of a positive treatment effect smaller than that specified. Similarly, the sample size provided limited power to explore certain clinical and immunological subgroups in whom benefit might be apparent. Finally, while

the timing of enrolment makes it unlikely that participants were infected with immune-evasive SARS-CoV-2 strains, the study is limited by the lack of data on viral strains in participants.

In summary, these results have implications beyond the hIVIG products studied here. CP was used widely early in the course of the COVID-19 pandemic. Based on the hypothesized antiviral effects of neutralizing antibodies hIVIG would be expected to confer greater and more consistent benefit: at the dose studied here these hIVIG products contain levels of neutralizing antibodies that are generally above those seen with high titer CP[16]. The overall lack of benefit, lack of any differential treatment effect by hIVIG product potency, and potential safety signal in nAb positive participants argue that CP is also unlikely to be providing benefit to hospitalized patients and raise concerns about harms in certain groups. It is noteworthy and an important lesson for both COVID-19 and future pandemics that there is no evidence of efficacy for CP or hIVIG among hospitalized patients[21].

Finally, while there was no evidence of clinical benefit in this hospitalized group when used with standard of care that includes remdesivir, a potential role for hIVIG may still be found in earlier disease stages of COVID-19 or special populations. As with other passive immunotherapies it is possible that a population treated very early in the onset of disease might benefit, as might groups with persistent failure to mount humoral immune responses to infection.

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Data Sharing Statement

Deidentified data from the ITAC trial will be made available one year following publication of final results from the trial. Supporting documents will be made available, including the protocol, statistical analysis plan, informed consent document, and data dictionary. Data will be made available to researchers after approval of a proposal for use of the data. Proposals for data use should be submitted using the Research Proposal Form on the INSIGHT website: www.insight-trials.org.

Contributors Statement

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Declaration of Interests

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