

Pharmacokinetics and Efficacy of Human Hyperimmune Intravenous Immunoglobulin Treatment of SARS-CoV-2 Infection in Adult Syrian Hamsters

Charles B. Stauff¹, Million Tegenge², Surender Khurana¹, Youri Lee¹, Prabhuanand Selvaraj¹, HanaGolding¹, Tony Wang¹, Basil Golding³.

¹ Division of Viral Products, Office of Vaccines Research and Review, ²Division of Clinical Evaluation and Pharmacology/Toxicology, Office of Tissues and Advanced Therapies,

³ Division of Plasma Protein Therapeutics, Office of Tissues and Advanced Therapies, Center for Biologics Evaluation and Research, Food and Drug Administration (FDA), Silver Spring, Maryland, U.S.A.

Corresponding Author: Basil Golding (basil.golding@fda.hhs.gov)

Summary: In a hamster model of SARS-CoV-2 infection hyperimmune immunoglobulin pharmacokinetics (PK) was altered depending on the viral load, possibly explaining why antibodies were ineffective in hospitalized Covid-19 patients suggesting that dosing should be adjusted to account for the viral load.

ABSTRACT

Background: Following the failure of antibody therapies in treating COVID-19 hospitalized patients we investigated the impact of viral replication on the pharmacokinetics (PK) and efficacy of a hyperimmune SARS-CoV-2 Immune Globulin (CoVIG) product in treatment of SARS-CoV-2 infection using the adult Syrian hamster model.

Methods: The CoVIG was manufactured from plasma donors who had recovered from COVID-19. The dose used (400 mg/kg) was based on the dose given in clinical trials to hospitalized COVID-19 patients. Hamsters were given a single dose of CoVIG two days after challenge with the SARS-CoV-2 virus (isolate NY/PV08410/2020), followed by sampling of blood, nasal, tracheal and lung tissues at different time points. The blood samples were assayed for anti-SARS-CoV-2 spike binding and used to calculate PK parameters. Nasal washes, trachea, and lung samples were assayed for viral replication by PCR (sgRNA).

Results: CoVIG-treated hamsters showed a reduction in viral replication in the lower respiratory tract, but minimally in the upper respiratory tract, following challenge with SARS-CoV-2. Challenge with SARS-CoV-2 resulted in altered PK parameters proportionate to viral replication, resulting in decreased **area under the curve (AUC)**, accelerated clearance and shorter half-life of CoVIG.

Conclusions: These data indicate that in the presence of actively replicating SARS-CoV-2 virus, PK parameters are altered and should trigger an adjustment in dosing of CoVIG.

Key Words: SARS-CoV-2, immune globulin, hamsters, pharmacokinetics, viral replication, Covid-19

Introduction

To date, the COVID-19 pandemic continues to claim lives. While limited anti-viral treatments can be provided early after SARS-CoV-2 exposure to individuals with high risk of severe disease. The potential benefits of convalescent plasma (CP) treatment have been investigated in clinical trials with mixed results. Patients treated early in the disease, who are seronegative may benefit from CP treatment. However, a large randomized controlled trial found that hospitalized patients with established disease did not benefit from CP treatment. **On the other hand, immunocompromized patients receiving convalescent plasma may benefit.** This led to modification of the FDA Emergency Use Authorization (EUA) for CP emphasizing the need for high neutralizing titer units administered early in the course of disease.

Hyperimmune SARS-CoV-2 Immune Globulin products (CoVIG) contain IgG at a 10-fold higher concentration than convalescent plasma and are released based on a SARS-CoV-2 neutralization titer not less than (1:320-1:640). Thus, it was anticipated that CoVIG products would provide better therapeutic benefit to hospitalized COVID-19 patients than CP. However, a Phase 3 clinical trial for treatment of SARS-CoV-2 inpatients (within 12 days of symptom onset) with CoVIG (ITAC), sponsored by the U.S. National Institute of Allergy and Infectious Diseases (NIAID), did not meet its primary endpoints (<https://intranet.grifols.com/documents/38081080/1025666635/np-20210402-en.pdf/2b639903-5415-477a-94f1-0c5938185074>).

Similar results were seen with monoclonal antibodies (MAbs), i.e. they were not effective in hospitalized COVID-19 patients. Subsequently, EUAs for the Eli Lilly and Regeneron MAb cocktail products excluded hospitalized patients with moderate or severe disease.

One explanation for the negative outcome in these trials is that the dose and frequency of antibody administered was insufficient to **deal with** the viral load in the hospitalized COVID-19 patients. Our hypothesis is that virus enhances clearance and reduces the half-life of therapeutic antibodies as they form immune complexes, are cleared rapidly by the reticulo-endothelial system.

This hypothesis was tested in the Syrian hamster challenge model. A single dose of CoVIG was administered intravenously to hamsters either unchallenged or two days after challenge with SARS-CoV-2 virus to assess the impact of virus on CoVIG PK parameters. While previous work has demonstrated the prophylactic benefit of intraperitoneally injected CP and MAbs in the hamster model, we have chosen to investigate the use of intravenously administered CoVIG on day 2 post challenge, which represents the day of peak viral

replication in this model , and more closely resembles the clinical trials of these therapeutic products. The study design allowed us to determine to what extent viral replication alters the PK. The dose used in our study (400 mg/kg) is identical to the dose used in the NIAID clinical trial. In addition to antibody concentration in the blood, we measured the impact of treatment on weight change, and viral replications in the nasal washes, trachea, and lungs of untreated and treated animals.

Materials and Methods

Cells and Viruses

Vero cells (ATCC CRL 1586) were maintained in DMEM medium (Gibco/Invitrogen Life Technologies, Carlsbad CA) supplemented with 10% fetal bovine serum (Hyclone).

The SARS-CoV-2 isolate NY/PV08410/2020 (NR-53514) was obtained from BEI Resources, NIAID, NIH, and had been passed three times in once in Vero E6 cells prior to acquisition, and once in our lab. The passaged virus was sequenced and verified to contain no mutations compared to its original seed virus.

Hyperimmune immunoglobulin from SARS-CoV-2 convalescent patients (CoVIG)

A batch of CoVIG prepared from 250-400 COVID-19 CP donors was purchased from a commercial company. The manufacturing processes of IVIG usually includes cold alcohol fractionation (Cohn-Oncley), anion-exchange and size-exclusion chromatography. The final product is sterile-filtered IgG ($\geq 95\%$) and formulated at 100 mg/mL. The pseudovirus neutralization 50% titer (PsVNA50) of this batch was 1:1240 as determined by pseudovirus neutralization assay (WA-1 spike) established in our laboratory .

Hamster Experiments

Cannulated (jugular vein) adult male Syrian hamsters (*Mesocricetus aurata*) were purchased from Envigo and housed in the vivarium at the White Oak campus of the FDA. All experiments involving SARS-CoV-2 challenge were performed within the biosafety level 3 (BSL-3) suite on the White Oak campus of the FDA. The animals were implanted

subcutaneously with IPTT-300 transponders (BMDS), randomized, and housed 2 per cage in sealed, individually ventilated rat cages (Allentown). Hamsters were fed irradiated 5P76 (Lab Diet) *ad lib*, housed on autoclaved aspen chip bedding with reverse osmosis-treated water provided in bottles, and were acclimatized for 4-6 days or more prior to the experiments. The study protocol details were approved by the White Oak Consolidated Animal Care and Use Committee and carried out in accordance with the PHS Policy on Humane Care & Use of Laboratory Animals. To measure PK, mock-infected or challenged hamsters (n=8) were given 400 mg/kg human intravenous immunoglobulin (CoVIG) 2 days post infection through the jugular vein. A mock-treated group (n=8) was challenged but did not receive CoVIG treatment. Cannulated hamsters were challenged with 10^5 PFU of NY/PV08410/2020 via the intranasal route. Nasal washes, and tracheal and lung tissue samples of infected animals were collected as described previously .

SARS-CoV-2 sub-genomic RNA Quantification

RNA was extracted from 50 μ l nasal washes or 0.1-gram tissue homogenates using QIAamp viral RNA mini kit or the RNeasy 96 kit (Qiagen) and eluted with 60 μ L of water. Five μ L RNA was used for each reaction in real-time RT-PCR. Quantification of SARS-CoV-2 E gene subgenomic mRNA (sgRNA) was conducted using Luna Universal Probe One-Step RT-qPCR Kit (New England Biolabs) on a Step One Plus Real-Time PCR system (Applied Biosystems). The primer and probe sequences were: SARS2EF: CGATCTCTTGATAGATCTGTTCT; PROBE: FAM-ACACTAGCCATCCTTACTGCGCTTCG- BHQ-1; SARS2ER: ATATTGCAGCAGTACGCACACA. To generate a standard curve, the cDNA of SARS-CoV-2 E gene sgRNA was cloned into a pCR2.1-TOPO plasmid. The copy number of sgRNA was calculated by comparing to the standard curve obtained with serial dilutions of the standard plasmid. The detection limit of the sgRNA was determined to be 25

copies/reaction. Values below detection limits were mathematically extrapolated based on the standard curves for graphing purpose.

Pharmacokinetic Analysis

Blood samples for PK analysis were collected before the CoVIG administration (time 0) and at 1 - 1.5, 4, 24, 48, 72, 96, and 120 hours following administration of 400 mg/kg of CoVIG (Figure 1). The blood concentration-time data of CoVIG were normalized to the initial observed concentrations within 1-1.5 hours and analyzed by non-compartmental analysis using Phoenix WinNonlin® version 8.3 (Certara L.P., Pharsight, St. Louis, MO). **The initial sampling time following infusion of CoVIG was variable (1 to 1.5 hours) and hence we normalized the concentrations based on the observed initial concentration. AUC, clearance, half-life and volume of distribution at steady state were derived as previously described for total CoVIG without baseline adjustment, due to the negligible level prior to infusion of CoVIG. Half-life was calculated by regression analysis on at least 3–4 concentration-time data points at the terminal phase of the log-linear plot.**

ELISA

Immulon plates (96 well) were coated with 100 ng/100 μ L of recombinant SARS-CoV-2 prefusion spike protein in PBS overnight at 4°C. Starting at a 1:100 dilution, serum samples were serially diluted 1:5 and applied to the protein-coated plate for 1 hour at ambient temperature. Serum samples were assayed in duplicates. Standard CoVIG concentrations were assayed along with the experimental hamster serum samples. After three washes with PBS/0.05% Tween 20, bound antibodies were detected with a donkey anti-human IgG Fc-specific HRP-conjugated antibody (Jackson Immuno Research, West Grove, Pennsylvania). After 1 hour, plates were washed and o-phenylenediamine dihydrochloride (ThermoFisher, Waltham, Massachusetts) was added for 10min. Absorbance was measured at 492nm (BioTek plate reader, Winooski, Vermont). The absorption from the known dilutions of the CoVIG was fitted to a standard curve using BioTek Gen5 software. Human IgG concentration was determined based on the absorbance values of the standard CoVIG samples.

Statistical Analysis

Statistical analyses were performed with GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA). We compared the treatment groups and controls using Student's t-test or one-way ANOVA with Tukey's multiple comparisons test. Differences between groups were considered to be significant at a *P* value of <0.05.

Results

CoVIG effect on viral replication

Adult Syrian hamsters were first challenged with SARS-CoV-2 virus to model treatment of patients with CoVIG following a known infection event and presentation of clinical symptoms. Mock-treated animals were challenged with 10^5 PFU of the SARS-CoV-2 isolate NY/PV08410/2020 (a B.1 variant containing D614G in the Spike) intranasally (Figure 1, Group 1). Unchallenged hamsters received 400 mg/kg human CoVIG (Figure 1, Group 2). A third group of animals was challenged with 10^5 PFU of the SARS-CoV-2 isolate NY/PV08410/2020 followed by a single dose of 400 mg/kg human CoVIG (Figure 1, Group 3) two days after challenge. Viral replication assays by qRT-PCR for sgRNA were performed on samples from upper (URT) and lower respiratory tracts (LRT) on days 5 and 7 post-infection (DPI). Rapid SARS-CoV-2 replication in the URT with peak sgRNA concentrations were observed 2 days post-infection (Figure 2C). After treatment with CoVIG, subsets (n=4) of each group were euthanized and organs collected to measure viral replication by qRT-PCR of sgRNA on days 5 and 7 post infection (Figure 2 A and B). Viral replication in the lungs was reduced by >10-fold in CoVIG -treated compared to untreated animals at 5 DPI, and by >50-fold at 7 DPI (Figure 2A). In the trachea, no statistically significant change in viral replication was noted between the treated vs. untreated animals, although the mean viral replication at day 5 post infection was lower in CoVIG-treated group than control-treated group (Figure 2B). Viral replication in nasal washes was reduced 10-fold only on day 3 following CoVIG treatment, but no significant differences were detectable at any other time point (Figure 2C). Concomitant with the reduced viral replication in the lungs at 7 DPI, recovery in CoVIG treated animals was seen to be quicker compared to mock-treated controls with significantly higher body weights observed at 6 and 7 DPI (Figure 2D).

SARS-CoV-2 effect on PK parameters

We tested whether SARS-CoV-2 infection affects the CoVIG PK parameters using the serum samples collected from the Syrian hamsters that were either mock-infected (Group 2) or challenged with 10^5 PFU of SARS-CoV-2 and then treated at 2 DPI with 400 mg/kg human CoVIG intravenously (Group 3). Sera were collected at specific time points (Fig. 1) and used to determine a concentration-time curve (Figure 3A). The same data were used to calculate AUC, clearance, and half-life in mock-infected controls and SARS-CoV-2 challenged hamsters (Figure 3B). A significant decrease (Student's t-test, $p=0.0380$) was observed in AUC for SARS-CoV-2 challenged hamster serum corresponding with a significant increase in CoVIG clearance (Figure 3B). A 2-fold decrease in hCoV-2IG half-life (Figure 3B) was observed in SARS-CoV-2 challenged hamsters, however, this difference was not statistically significant (Student's t-test, $p=0.0739$). **In general, there is high uncertainty in the estimation of half-life for IGIV products presumably due to a limitation of the PK sampling schedule. In the present study, we observed a high variability in the calculated half-life and in some cases the half-life ranges up to the last sampling time point of 120 hours. These results suggest that additional PK samples at later time points may be needed to reduce the uncertainty.**

The hamsters challenged with SARS-CoV-2 were further divided into two groups based on nasal viral replication (≤ 3.5 vs. > 3.5 \log_{10} sgRNA copies) (Figure 4A). In animals with low nasal viral replication the CoVIG clearance rate was not significantly different from untreated control animals. But for animals with high SARS-CoV-2 viral replication the **difference in** clearance rate compared with untreated control animals reached statistical significance ($p=0.01$) (Figure 4A and B). **As shown in Figure 4B, 3 of 5 animals with higher viral load (60%) have clearly higher clearance values as compared to the clearance values of animals with low viral load. However, the overall sample size is small. The overall correlation between the CoVIG clearance rate and SARS-CoV-2 viral replication was highly significant ($p=0.0026$) (Fig. 4C).** Higher nasal viral replication was associated with 20-55% higher CoVIG clearance rates, 14-33% lower systemic exposure (AUC) and 19% shorter half-life of CoVIG in the blood compared to animals with lower viral replication (Figure 4D). These results indicate that higher viral replication may lead to more rapid antibody clearance of immunoglobulin products against COVID-19.

Discussion

Despite the disappointing results in the INSIGHT-013T study, CoVIG will probably be further evaluated as post exposure-prophylaxis treatment in certain populations at high risk for developing severe COVID disease. Therefore, it is important to identify parameters that may impact the IgG levels and clearance rates. These may include host (e.g. body weight, Ig distribution, and Ig catabolism) and viral parameters (i.e. viral replication in URT and LRT). The important goal is to reach stable trough levels of CoVIG to assure that virus has been successfully cleared so that clinical outcomes are improved in COVID-19 patients.

In the current study, we assessed whether viral replication affected PK parameters after administration of CoVIG to Syrian hamsters with and without challenge with SARS-CoV-2 two days earlier. Compared with unchallenged animals, challenged animals showed decreased CoVIG in the concentration-time curves. The calculated AUC was decreased, the clearance was increased, and the half-life shortened. The greater the viral replication the more marked were the changes in plasma clearance of CoVIG. These findings indicate that viral presence does influence CoVIG concentration negatively and may explain the relatively poor outcome after treatment of established COVID-19 infection with CP, hyperimmune SARS-CoV-2IG and monoclonal antibodies (Eli Lilly <https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-monoclonal-antibodies-treatment-covid-19-0>);

Regeneron: <https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-monoclonal-antibodies-treatment-covid-19>). The results also underscore the importance of monitoring antibody and viral levels during antibody treatment in infected individuals. This could lead to better understanding of the viral load-antibody response relationship and allow for more optimal dosing of antibody-based therapeutics for treatment of COVID-19.

Importantly, in the context of this hamster study, CoVIG did reduce viral replication in the lungs as measured by PCR of viral sgRNA. The drop in nasal wash viral sgRNA was

limited to the day after treatment and agrees with published work . Since virus replication in the URT is vital for transmission , the inability to reduce viral replication in the URT implies that CoVIG treatment may not reduce viral transmission, but could shorten symptomatic disease.

Hyperimmune globulin products have been used to treat infectious disease for several decades with some success . Experience with these products has revealed that prophylaxis, using IG against hepatitis A virus during outbreaks can be highly effective . On the other hand, treatment of established disease is less likely to be effective. Even in the realm of monoclonal antibodies, whose potency may be more than 1000-fold greater than polyclonal antibodies, FDA approval for established infections is relatively rare. An exception is a mixture of MAb used to treat Ebola, which reduces mortality from 51% to 34% (<https://www.fda.gov/news-events/press-announcements/fda-approves-first-treatment-ebola-virus>).

Hepatitis B immune globulin (HBIG) is approved for post-exposure prophylaxis after needle sticks and to prevent disease in the newborn of infected mothers [NABI HBIG Package Insert, <https://www.fda.gov/media/74707/download>]. Interestingly, liver transplant patients, who were HBsAg positive did respond to HBIG treatment which prevented recurrence of hepatitis B in the transplanted liver . However, 15-fold higher dose, repeated multiple times was required to achieve adequate trough levels and patients becoming HbsAg negative . Moreover, patients with replicating virus (E-antigen positive) had higher HBIG clearance than E-antigen negative patients. Similarly, PK studies in HIV-infected patients showed lower AUC and higher clearance in subjects who were HIV antigen positive compared to those who were HIV antigen negative . These studies underscore the need to take viral load and PK into account when treating active viral disease with hyperimmune IG products .

Target-mediated drug disposition (TMDD) of MAbs include Rituximab treatment of B cell lymphoma and daratumumab therapy for multiple myeloma . In both cases high doses and frequent administration were required to obtain tumor remission. PK assessment showed a correlation between remission and achievement of stable trough serum antibody levels. While tumor antigen load is not easy to assess, treatment dose and frequency could be based on attainment of stable blood trough antibody levels. This approach was not adopted in the CP, hyperimmune SARS-CoV-2IG, and MAb trials in hospitalized COVID-19 patients.

Sub-optimal antibody dosing can lead to SARS-CoV-2 escape variants as documented by *in vitro* studies showing the propensity for viral escape mutants to develop in the presence of SARS-CoV-2 neutralizing antibodies . Additionally, low dose treatment could be associated with antibody-dependent enhanced disease, as reported for dengue .

Although vaccination has been highly successful in curbing COVID-19 there are individuals that do not respond to vaccination because of a compromised immune system (primary immunodeficiency, hematologic and solid organ malignancies, transplant recipients and patients with autoimmune disease on immunosuppressive therapies such as steroids and mycophenolate) . **For vaccine non-responders and high risk immunosuppressed**

individuals it is important to consider CoVIG as prophylaxis or post-exposure treatment, which likely will be more reliable than convalescent plasma treatment in terms of higher neutralizing titers and broader reactivity against variants of concern .

Limitations of this study relate to the utility of the hamster model in predicting human responses. Although hamsters are susceptible to SARS-CoV-2 infection, they exhibit mild disease and may not respond identically to treatments. Nevertheless, we were able to detect a treatment effect with the human CoVIG product. Additionally, due to species differences, human antibodies given to hamsters are expected to elicit hamster anti-human response that ultimately clear the human IgG quicker than humans. However, in the current study the CoVIG treatment took place on day 2 post challenge and prior to generation of an immune response against the human antibodies. Importantly, the PK values were compared between hamsters with and without viral challenge showing that higher viral loads altered the PK parameters, namely increased clearance and decreased half-life.

The results of this study suggest that measurements of PK in early COVID-19 patients treated with CoVIG should be undertaken for dosing adjustment to ensure that stable antibody trough levels are reached and maintained to achieve a beneficial impact on COVID-19.

Accepted Manuscript

NOTES

Acknowledgements: We thanks Drs. Evi Struble and Jennifer Reed for careful review and helpful comments.

Disclaimer: These findings are an informal communication and represent the authors' best judgment. These comments do not bind or obligate the U.S. FDA. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Funding: The work described in this manuscript was partially supported by FDA Medical Countermeasures Initiative (MCMi) grant # OCET 2021-1565 and NIH-NIAID IAA #AAI20040, and internal grants from U.S. FDA. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest: None to report

Accepted Manuscript

REFERENCES

1. Casadevall A, Joyner MJ, Pirofski LA. A Randomized Trial of Convalescent Plasma for COVID-19-Potentially Hopeful Signals. *JAMA* **2020**; 324(5): 455-7.
2. 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.
3. Bloch EM, Shoham S, Casadevall A, et al. Deployment of convalescent plasma for the prevention and treatment of COVID-19. *J Clin Invest* **2020**; 130(6): 2757-65.
4. Group RC. Convalescent plasma in patients admitted to hospital with COVID-19 (RECOVERY): a randomised controlled, open-label, platform trial. *Lancet* **2021**; 397(10289): 2049-59.
5. Senefeld JW, Klassen SA, Ford SK, et al. Use of convalescent plasma in COVID-19 patients with immunosuppression. *Transfusion* **2021**; 61(8): 2503-11.
6. Bournazos S, Gupta A, Ravetch JV. The role of IgG Fc receptors in antibody-dependent enhancement. *Nat Rev Immunol* **2020**; 20(10): 633-43.
7. Selvaraj P, Lien CZ, Liu S, et al. SARS-CoV-2 infection induces protective immunity and limits transmission in Syrian hamsters. *Life Sci Alliance* **2021**; 4(4).
8. Stauff CB, Lien CZ, Selvaraj P, Liu S, Wang TT. The G614 pandemic SARS-CoV-2 variant is not more pathogenic than the original D614 form in adult Syrian hamsters. *Virology* **2021**; 556: 96-100.
9. Nunez IA, Lien CZ, Selvaraj P, et al. SARS-CoV-2 B.1.1.7 Infection of Syrian Hamster Does Not Cause More Severe Disease, and Naturally Acquired Immunity Confers Protection. *mSphere* **2021**: e0050721.
10. Haagmans BL, Noack D, Okba NMA, et al. SARS-CoV-2 Neutralizing Human Antibodies Protect Against Lower Respiratory Tract Disease in a Hamster Model. *J Infect Dis* **2021**; 223(12): 2020-8.
11. Tang J, Ravichandran S, Lee Y, et al. Antibody affinity maturation and plasma IgA associate with clinical outcome in hospitalized COVID-19 patients. *Nat Commun* **2021**; 12(1): 1221.
12. Ravichandran S, Coyle EM, Klenow L, et al. Antibody signature induced by SARS-CoV-2 spike protein immunogens in rabbits. *Sci Transl Med* **2020**; 12(550).
13. Mahmood I, Tegenge MA, Golding B. Considerations for pharmacokinetic assessment of immunoglobulins: Gammagard in very low birth weight neonates with and without baseline-correction. *Int Immunopharmacol* **2020**; 82: 106358.
14. Tegenge MA, Mahmood I, Struble E, Golding B. Dosing Considerations for Antibodies Against COVID-19. *Drugs R D* **2021**; 21(1): 1-8.
15. 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.
16. Laporte M, Raeymaekers V, Van Berwaer R, et al. The SARS-CoV-2 and other human coronavirus spike proteins are fine-tuned towards temperature and proteases of the human airways. *PLoS Pathog* **2021**; 17(4): e1009500.
17. Casadevall A, Pirofski LA, Joyner MJ. The Principles of Antibody Therapy for Infectious Diseases with Relevance for COVID-19. *mBio* **2021**; 12(2).
18. Wasley A, Fiore A, Bell BP. Hepatitis A in the era of vaccination. *Epidemiol Rev* **2006**; 28: 101-11.
19. Dickson RC, Terrault NA, Ishitani M, et al. Protective antibody levels and dose requirements for IV 5% Nabi Hepatitis B immune globulin combined with lamivudine in liver transplantation for hepatitis B-induced end stage liver disease. *Liver Transpl* **2006**; 12(1): 124-33.
20. Fletcher CV, Goodroad BK, Cummins LM, Henry K, Balfour HH, Jr., Rhame FS. Pharmacokinetics of hyperimmune anti-human immunodeficiency virus immunoglobulin in persons with AIDS. *Antimicrob Agents Chemother* **1997**; 41(7): 1571-4.

21. Rozman S, Grabnar I, Novakovic S, Mrhar A, Jezersek Novakovic B. Population pharmacokinetics of rituximab in patients with diffuse large B-cell lymphoma and association with clinical outcome. *Br J Clin Pharmacol* **2017**; 83(8): 1782-90.
22. Xu XS, Yan X, Puchalski T, et al. Clinical Implications of Complex Pharmacokinetics for Daratumumab Dose Regimen in Patients With Relapsed/Refractory Multiple Myeloma. *Clin Pharmacol Ther* **2017**; 101(6): 721-4.
23. Greaney AJ, Starr TN, Barnes CO, et al. Mapping mutations to the SARS-CoV-2 RBD that escape binding by different classes of antibodies. *Nat Commun* **2021**; 12(1): 4196.
24. Katzelnick LC, Gresh L, Halloran ME, et al. Antibody-dependent enhancement of severe dengue disease in humans. *Science* **2017**; 358(6365): 929-32.
25. Rabinowich L, Grupper A, Baruch R, et al. Low immunogenicity to SARS-CoV-2 vaccination among liver transplant recipients. *J Hepatol* **2021**; 75(2): 435-8.
26. Connolly CM, Boyarsky BJ, Ruddy JA, et al. Absence of Humoral Response After Two-Dose SARS-CoV-2 Messenger RNA Vaccination in Patients With Rheumatic and Musculoskeletal Diseases: A Case Series. *Ann Intern Med* **2021**.
27. Rincon-Arevalo H, Choi M, Stefanski AL, et al. Impaired humoral immunity to SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients and dialysis patients. *Sci Immunol* **2021**; 6(60).
28. Tang J, Lee Y, Ravichandran S, et al. Epitope diversity of SARS-CoV-2 hyperimmune intravenous human immunoglobulins and neutralization of variants of concern. *iScience* **2021**: 103006.

Figure Legends:

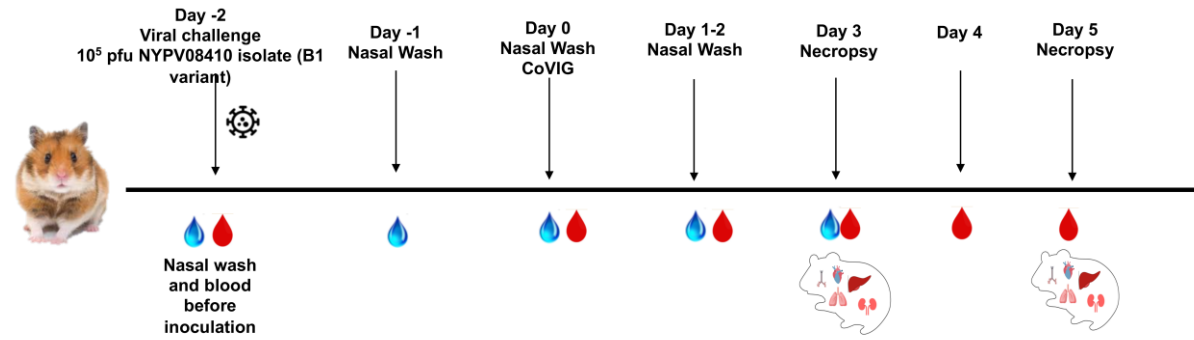
Figure 1: Experimental Design. This schema shows the time course of viral challenge, CoVIG treatment, and sampling times of blood and tissues. The three different groups of hamsters are also defined.

Figure 2: Efficacy of human IVIG in treating SARS-CoV-2 infection in adult Syrian hamsters. Levels of viral replication in lung (A) and trachea (B) homogenates along with nasal wash (C) samples. Morbidity in treated (CoVIG) and control (saline) animals was also measured using body weight (D). The arrow indicates the day that hamsters (n=8) were treated with 400 mg/kg CoVIG. DPI: Days post-infection. CoVIG: hyperimmune human SARS-CoV-2 immune globulin.

Figure 3: Blood concentration-time data and PK parameters for control and SARS-CoV-2 alpha strain virus challenged hamsters. (A). Rapid clearance of CoVIG from the blood in challenged animals (red diamond) compared to the controls (blue circle) (B). Summary of CoVIG PK parameters for control and challenged hamsters.

Figure 4: Correlation analysis of nasal viral replication versus PK parameters. (A) A cluster of nasal viral replication at day 3 post-infection as low ($\leq 3.5 \log_{10}$ sgRNA copies) and high ($> 3.5 \log_{10}$ sgRNA copies). (B) High nasal viral replication is significantly associated with clearance of CoVIG from the blood. (C) Correlation analysis of nasal viral replication (\log_{10} sgRNA copies) versus clearance, $R^2=0.55$. (D) About 20-55 % higher clearance, 14-33% lower systemic exposure and 19% shorter half-life in animals with higher viral replication versus lower viral replication.

Figure 1



Group 1	Mock-Treated (Saline) (n=8), Challenged
Group 2	CoVIG product at 400 mg/kg (n=8)
Group 3	CoVIG product at 400 mg/kg (n=8), Challenged

Figure 2

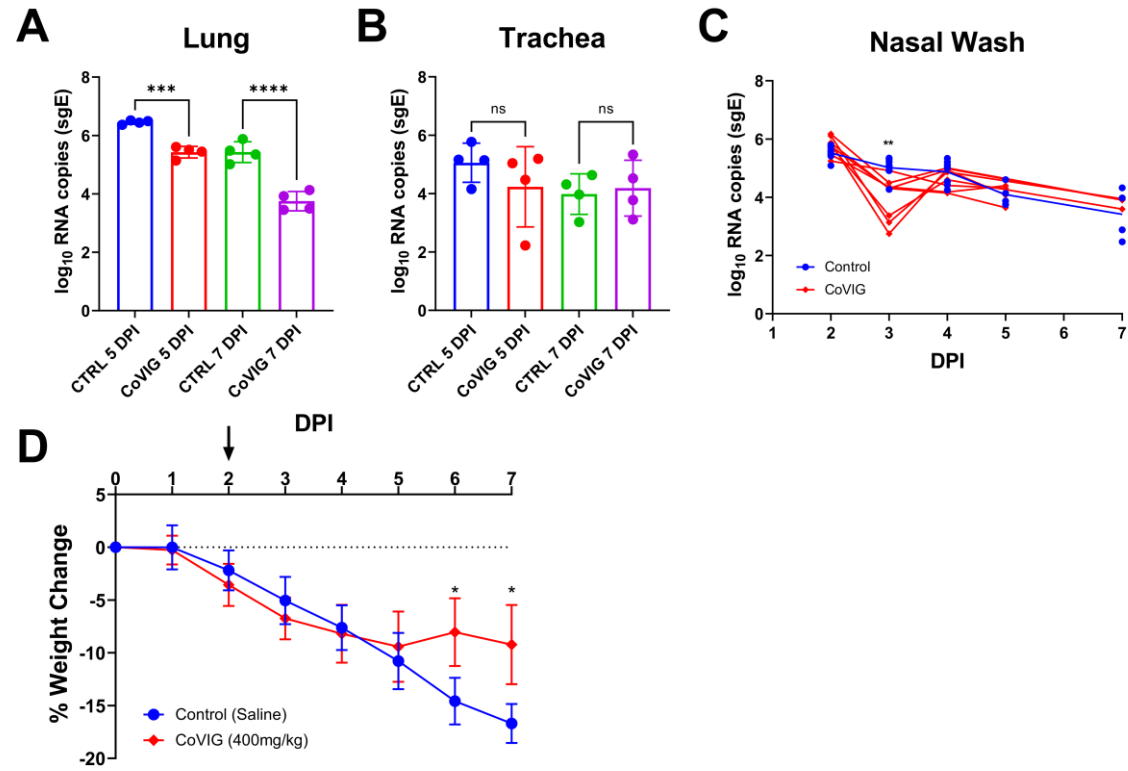
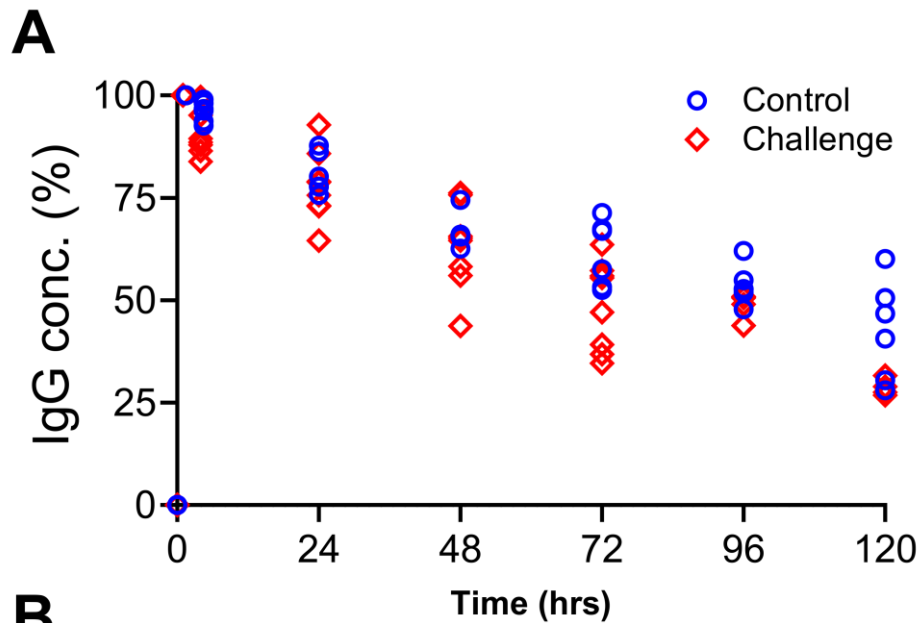


Figure 3



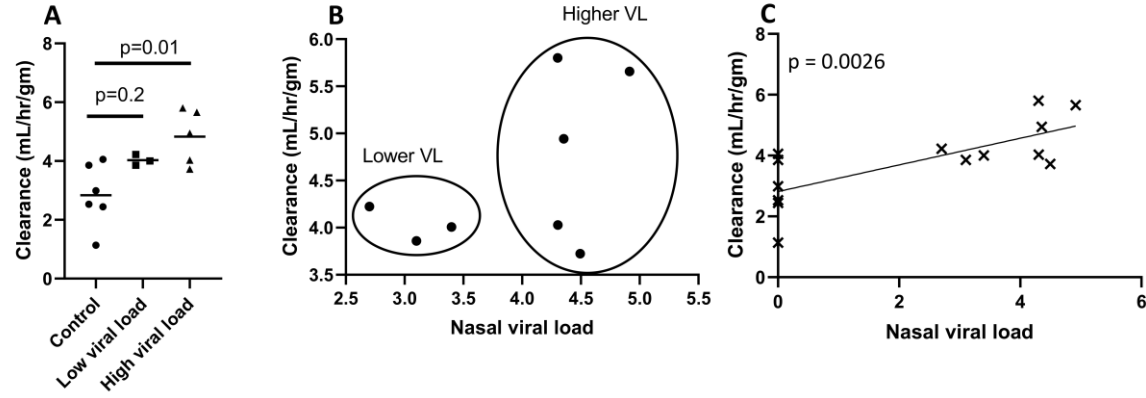
B

	Control (n =6)	Challenge (n =8)	% Mean Difference
AUC _(0-last hr) (% x hr)	7944 ± 260	6169 ± 1681	-22
AUC _(0-∞) (% x hr)	16817 ± 9367	9069 ± 1506	-46
Clearance (mL/hr/gm)*	2.8 ± 1.1	4.5 ± 0.8	61
Clearance (mL/hr/gm)**	5.0 ± 0.2	6.9 ± 1.9	38
Half-life(hr)	124 ± 95	58 ± 13	-53
V _{ss} (mL/gm)	406 ± 70	379 ± 53	-7

*Calculated based on AUC_(0-∞), **calculated based AUC_(0-last hr).

ACQ

Figure 4



	Low nasal viral load (n=3)	High nasal viral load (n=5)	% Mean Difference
AUC _(0-last hr) (% x hr) ^a	7773 ± 432	5206 ± 1328	-33
AUC _(0-∞) (%x hr)	9940 ± 449	8546 ± 1720	-14
Clearance (mL/hr/gm) ^b	4.0 ± 0.2	4.8 ± 0.9	21
Clearance (mL/hr/gm) ^c	5.2 ± 0.3	8.0 ± 1.6	55
V _{ss} (mL/gm)	328 ± 37	409 ± 35	24

^aIgG concentrations were percent of the initial observed concentrations within 1-1.5 hours.

^bCalculated based on AUC_(0-∞), ^ccalculated based on AUC_(0-last hr).