1 **SARS-CoV-2 hyperimmune intravenous human immunoglobulins neutralizes**

2 **Omicron subvariants BA.1, BA.2, BA.2.12.1, BA.3 and BA.4/BA.5 for treatment of**

3 **COVID-19**

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ABSTRACT

Our study demonstrates that neither 2020-convalescent plasma (CP) nor 2019/2020-immunoglobulin (IVIG) neutralize Omicron subvariants BA.1 to BA.5. In contrast, hyperimmune 2020-hCoV-2IG lots neutralized Omicron VOCs, similar to 2022-CP from BA.1 breakthrough infections. Therefore, high-titer hCoV-2IG and CP could be evaluated for treatment of high-risk individuals infected with circulating Omicron subvariants.

INTRODUCTION

Since the start of SARS-CoV-2 pandemic, there has been wide use of convalescent plasma (CP) from COVID-19 recovered individuals as treatment for COVID-19 patients, especially those with immunocompromising conditions. However, low effectiveness was reported and was partially linked to the high variability in neutralizing activity of CP[1, 2]. The emergency use authorization was thus revised to recommend the use of only high-titer CP for treatment of COVID-19 patients.

Immunoglobulin products (IGs) are manufactured from pooled human plasma and contain more than 95% unmodified immunoglobulin G (IgG), which has intact Fc-dependent effector functions. IGs are widely used for the treatment of immunodeficiency syndromes. However, there are many off-label uses of IGs for several autoimmune and neurological conditions [3]. Most IGs are administered intravenously and are called IVIG.

Hyperimmune anti-SARS-CoV2 IVIGs (hCoV-2IG) are manufactured by fractionation of pooled plasma units and contain IgG at 10-fold higher concentration than in CP [4]. hCoV-2IG are being evaluated in clinical trials for treatment of COVID-19 [5]. The rapidly evolving SARS-CoV-2 Omicron sublineages (BA.1, BA.1.1, BA.2, BA.2.12.1, BA.3, BA.4 and BA.5) contain multiple mutations in the spike protein, which enhance transmissibility and escape neutralization by multiple neutralizing monoclonal antibodies approved for treatment of immunocompromised individuals[6].

To evaluate the preventive/therapeutic potential of polyclonal CP and hCoV-2IG against currently circulating SARS-CoV-2 Omicron subvariants, we measured the neutralization activity of 8 hCoV-2IG lots manufactured in 2020 against the ancestral

SARS-CoV-2 WA1/2020 strain as well as Omicron BA.1, BA.1.1, BA.2, BA.2.12.1, BA.3 and BA.4/BA.5 variants of concern (VOCs). Additionally, 14 IVIG preparations that were manufactured in 2019 (2019-IVIG) before the COVID-19 pandemic and 14 IVIG lots made from plasma donations in 2020 (2020-IVIG) were evaluated. For comparison, we evaluated 10 CP from recovered COVID-19 patients in early 2020 (2020-CP) and 6 CP from Omicron breakthrough infections in 2022 (2022-CP).

MATERIALS AND METHODS

Samples and study design

IVIG products approved by the FDA are polyclonal antibodies made from 10,000 or more U.S. plasma donors and may include cold alcohol fractionation (Cohn-Oncley), anion-exchange and size-exclusion chromatography. The final product is sterile-filtered IgG (> 95%) and formulated at 100 mg/mL. Fourteen intravenous immunoglobulin (2019-IVIG) batches were produced from plasma collected prior to August 2019 and 14 IVIG lots (each lot derived from >10,000 donors) made from plasma donations in 2020 and manufactured between October 2020 and January 2021 (2020-IVIG), were obtained from five manufacturers.

Eight hCoV-2IG batches prepared from large number of COVID-19 CP (~200-1000 donors per lot) were obtained/purchased from three commercial companies for blinded antibody analysis. The plasma units used in the manufacturing of the hCoV-2IG batches were collected in 2020 prior to emergence of the Delta and Omicron VOCs.

Ten random CP lots were obtained from recovered COVID-19 patients between May-September 2020 (at least 30-days post-recovery). At the time of collection SARS-

CoV-2 D614G was predominant strain in the US. Six CP lots were collected in February 2022 from recovered individuals following Omicron BA.1 breakthrough infections (in December 2021), who received at least two doses of mRNA vaccination.

Neutralization assay

Samples were evaluated in a qualified SARS-CoV-2 pseudovirion neutralization assay (PsVNA) using SARS-CoV-2 WA1/2020 strain and Omicron subvariants: BA.1, BA.1.1, BA.2, BA.2.12.1, BA.3 and BA.4/BA.5. The mutations in spike protein of these Omicron subvariants are shown in Supplementary Table S1. SARS-CoV-2 neutralizing activity measured by PsVNA correlates with PRNT (plaque reduction neutralization test with authentic SARS-CoV-2 virus) in previous studies [7, 8].

Neutralization assays were performed as previously described [8, 9]. Briefly, 50 µL of SARS-CoV-2 S pseudovirions (counting ~200,000 relative light units) were pre-incubated with an equal volume of medium containing serial dilutions (starting at 1:10) of all samples at room temperature for 1h. Then 50 µL of virus-antibody mixtures were added to 293T-ACE2-TMPRSS2 cells (10^4 cells/50 µL) in a 96-well plate. The input virus with all SARS-CoV-2 strains were the same (2×10^5 relative light units/50 µL/well). After a 3 h incubation, fresh medium was added to the wells. Cells were lysed 24 h later, and luciferase activity was measured using One-Glo luciferase assay system (Promega). The assay of each sample was performed in duplicate, and the 50% neutralization titer was calculated using Prism 9 (GraphPad Software). The limit of detection for the neutralization assay is 1:20. Two independent biological replicate

1 experiments were performed for each sample and variation in PsVNA50 titers was

2 <10% between replicates.

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4 **Quantification and statistical analysis**

5 Descriptive statistics were performed to determine the geometric mean titer

6 values and were calculated using GraphPad. All experimental data to compare

7 differences among groups were analyzed using lme4 and emmeans packages in R. To

8 ensure robustness of the results, absolute measurements were log2-transformed before

9 performing the analysis. Correlation and regression analyses were performed by

10 computing Spearman's rank correlation coefficient and significance in GraphPad Prism.

11

12 **RESULTS**

13 All 14 2019-IVIG lots had no neutralizing antibodies against either the ancestral

14 SARS-CoV-2 strain (WA1/2020) or the Omicron sub-linages (Supplementary Table S2).

15 Eleven out of the fourteen 2020-IVIG lots had very low PsVNA50 titers against

16 WA1/2020 (1:20.7-1:62.1) and no detectable neutralizing antibodies against the six

17 Omicron subvariants (Figure 1A and Supplementary Table S2), suggesting that a very

18 small fraction of the pooled plasma units came from SARS-CoV-2 seropositive donors.

19 Ten individual COVID-19 CPs (from 2020) collected prior to the emergence of

20 Omicron and its subvariants, showed heterogeneity in their neutralization titers against

21 WA1/2020. One CP lot was negative, and the 50% neutralization titers (PsVNA50) of

22 the other nine CP lots ranged from 1:68 to 1:2972 [geometric mean titer (GMT): 1:130]

23 (Figure 1A). For all 2020-CP lots, the titers against Omicron BA.1 were reduced by ~10-

fold compared with WA1/2020, with 8 of 10 CP lots having PsVNA50 titers less than

1:20. Only one CP lot showed minimal neutralizing titers of 1:78, 1:81, 1:61, 1:78, 1:53,

and 1:95 against Omicron subvariants BA.1, BA.1.1, BA.2, BA.2.12.1, BA.3, and

BA.4/BA.5, respectively. In contrast, CPs collected recently from Omicron BA.1

breakthrough infections in fully mRNA vaccinated individuals (2022-CP), exhibited

higher neutralization titers against the ancestral WA1 strain (GMT 435-3854).

Importantly, high neutralizing titers were observed against Omicron BA.1, BA1.1 and

BA.2, with reduction in neutralizing antibodies against Omicron subvariants BA.2.12.1,

BA.3, and BA.4/BA.5 (Supplementary Table S2 and Fig. 1A).

In contrast to the 2020-IVIG lots, all eight 2020-hCoV-2IGs lots demonstrated

high neutralization titers against the WA1/2020, ranging between 1:1742 and 1:7303

(GMT of 3319). Importantly, all 2020-hCoV-2IGs demonstrated cross-neutralizing

activity against Omicron subvariants (Supplementary Table S2 and Figure 1A). The

neutralization titers against BA.1, BA 1.1, and BA.2 were variable, with one lot (hCoV-

2IG-7) exhibiting high neutralization titers across all variants (>1:1000), two lots had low

titers (<1:100) and five lots had medium neutralization titers (≥1:100 to <1:235). The

neutralizing antibody titers against BA.2.12.1, BA.3, and BA.4/BA.5 for all hCoV-2IG lots

trended lower ranging from 1:10 to 1:753. The fold reduction in GMT of the Omicron

subvariants compared with WA1 ranged between 14.7 (BA.1) and 42 (BA.4/BA.5).

Correlation was observed between PsVNA50 against WA1/2020 and various Omicron

subvariants (p<0.0001) (Figure 1B), suggesting that higher neutralization titers against

the ancestral WA1/2020 strain could predict the neutralization activity against the

Omicron subvariants.

DISCUSSION

Our study demonstrates that despite a significant drop in PsVNA50 titers against the Omicron subvariants, the majority of 2020-hCoV2-IG lots demonstrated neutralization of BA.1, BA.1.1, BA.2, BA.2.12.1, BA.3, BA.4 and BA.5, at levels predicted to provide at least some protection against COVID-19 with circulating Omicron VOCs [10]. Non neutralizing antibodies may also provide some protection *in vivo* via Fc-mediated functions [11, 12]. However, Fc-effector activity in CP from multiple infections (including BA.1) was shown to be much reduced against BA.4. These findings are important since these lots of hCoV-2IG were manufactured with CP units collected prior to the appearance of Omicron. Therefore, high titer polyclonal antibodies against the ancestral strain in hCoV-2IG contained cross-reactive antibodies against subsequently emerging VOCs[13]. The titer of anti-Omicron neutralizing antibodies observed with the 2020-hCoV-2IG lots was 2-5-fold lower than the GMT observed with recent 2022-CP from Omicron BA.1 breakthrough infections, except for BA.4/BA.5. The concentration of IgG in CP (10mg/mL) is 10-fold lower than that in hCoV-2IG or IVIG (100mg/mL). However, the transfused volume per person ranges between 250-400 ml for both CP and IVIG preparations. Neutralizing antibody titers in the patients receiving these products would reflect these differences, i.e., lower titers if they received CP vs. hCoV-2IG or IVIG.

New hCoV-2IG batches could be generated from plasma donors who recently recovered from SARS-CoV-2 BA.4/5 infections or from vaccinated/infected individuals. While there are logistical challenges to hyperimmune globulin production (e.g., long lead time), hCoV-2IG have notable advantages over CP, including standardization of dose, pathogen reduction, and measurements of anti-SARS-CoV-2 neutralizing titers prior to release.

In conclusion, currently available hCoV-2IG lots from 2020 could be used immediately for treatment of high-risk individuals infected with Omicron subvariants. Our data predicts that future IVIG lots may show increasing anti-SARS-CoV-2 titers, since most plasma donors in the US are seropositive [14]. Newly manufactured hCoV-2IG lots should be screened for high neutralization activity against circulating SARS-CoV-2 variants, which will likely improve their effectiveness against COVID-19.

NOTES:

1 **Ethics Statement**

2   This study was approved by the Food and Drug Administration's Research

3 Involving Human Subjects Committee (RIHSC #2020-04-02). This study complied with

4 all relevant ethical regulations for work with human participants. Samples were collected

5 from adult subjects who provided informed consent to participate in the study. All

6 assays performed fell within the permissible usages in the original informed consent.

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15 **Conflict of Interest:** The authors declare that they have no competing interests.

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1 **Figure Legend**

2

3 **Figure 1: Neutralization of SARS-CoV-2 WA1/2020 strain and Omicron**

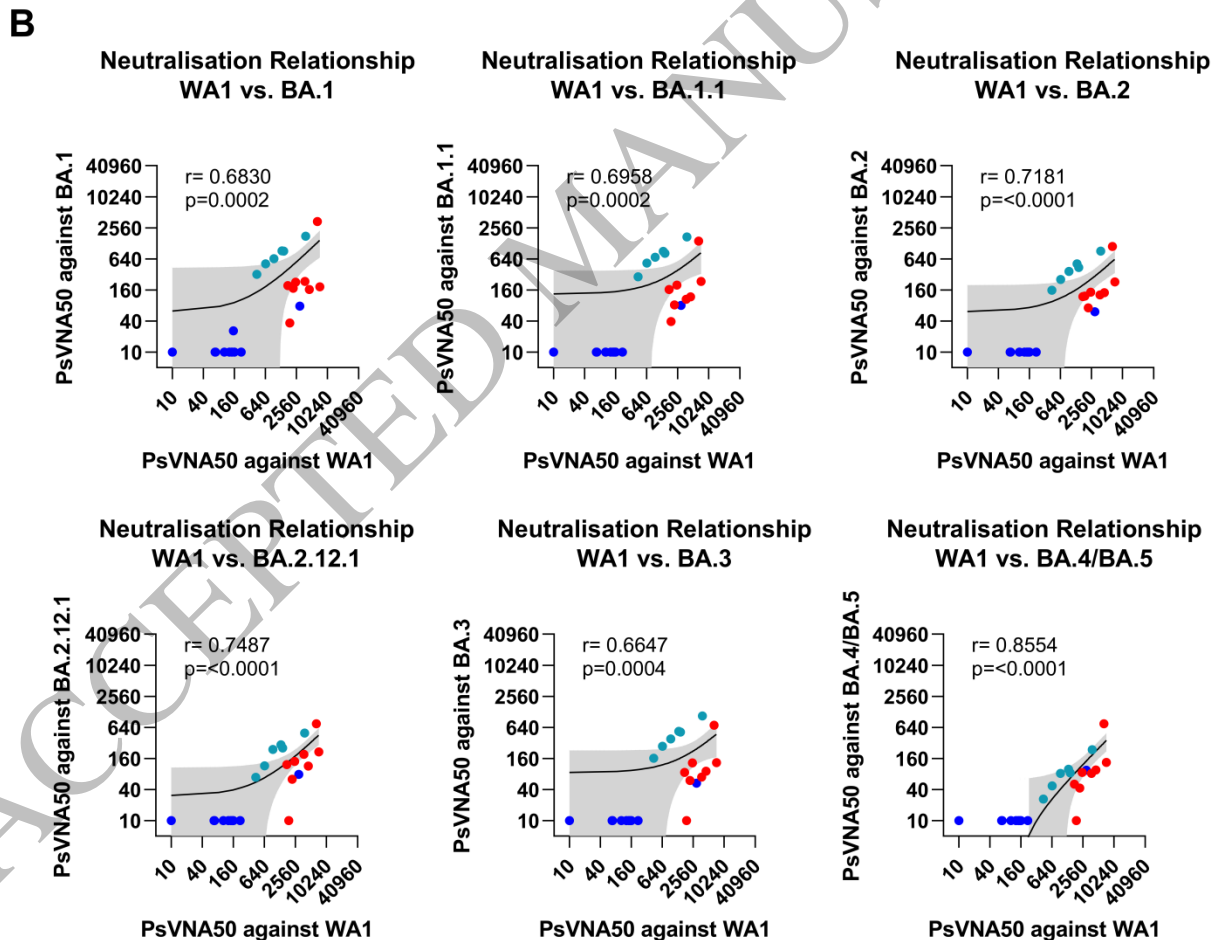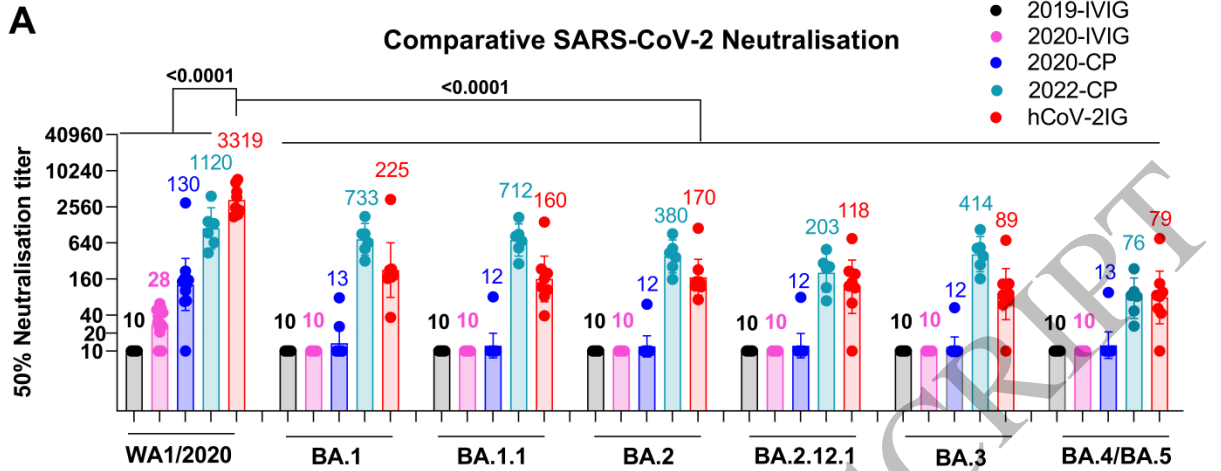4 **sublineages by IVIG, convalescent plasma and hCoV-2IG.**

5 (A) SARS-CoV-2 neutralization assays were performed by using pseudoviruses

6 expressing the SARS-CoV-2 spike proteins of the WA1/2020 vaccine strain or the

7 Omicron subvariants BA.1, BA.1.1, BA.2, BA.2.12.1, BA.3, BA.4 and BA.5 in 293-ACE2-

8 TMPRSS2 cells. SARS-CoV-2 neutralizing antibody titers was determined in each of the

9 control pre-pandemic 2019-IVIG (n =14; in black), 2020-IVIG (n =14; in pink), 2020

10 convalescent plasma (2020-CP; n = 10; in blue), 2022 convalescent plasma (2022-CP;

11 n = 6; in turquoise) and hCoV-2IG (n = 8; in red) preparations. The assay of each

12 sample was performed in duplicate to determine the 50% neutralization titer (PsVNA50).

13 Each data point represents an individual sample (circles) and indicates the 50%

14 neutralization titer obtained with each sample against the indicated pseudovirus. The

15 heights of the bars and the numbers over the bars indicate the geometric mean titers,

16 and the whiskers indicate 95% confidence intervals. The horizontal dashed line

17 indicates the limit of detection for the neutralization assay (PsVNA50 of 20). The raw

18 data and information regarding the samples and 50% neutralization titers against

19 SARS-CoV-2 strains are summarized in Supplementary Table S2. Differences between

20 SARS-CoV-2 strains were analyzed by lme4 and emmeans packages in R using

21 Tukey's pairwise multiple comparison test and the p-values are shown. (B) Relationship

22 of neutralizing antibodies against SARS-CoV-2 WA1/2020 and Omicron subvariants.

23 Correlation of SARS-CoV-2 WA1/2020 neutralizing titer versus Omicron subvariant

neutralizing titer for 2020 convalescent plasma (2020-CP; n = 10; in blue), 2022 convalescent plasma (2022-CP; n = 6; in turquoise) and hCoV-2IG (n = 8; in red) preparations. The black line in the scatter plots depict the linear fit of log2 transformed PsVNA50 values with shaded area showing 95% confidence interval. Correlations show spearman rank correlation coefficients and two-tailed P values.

Figure 1
165x205 mm (0.9 x DPI)