Increased Resistance of SARS-CoV-2 Variant P.1 to Antibody Neutralization

Pengfei Wang[1*], Maple Wang[1], Jian Yu[1], Gabriele Cerutti[2], Manoj S. Nair[1], Yaoxing Huang[1], Peter D. Kwong[2,3], Lawrence Shapiro[1,2], & David D. Ho[1,4,5,*]

[1]Aaron Diamond AIDS Research Center, Columbia University Vagelos College of Physicians and Surgeons, New York, NY, USA. [2]Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY, USA. [3]Vaccine Research Center, National Institutes of Health, Bethesda, MD, USA. [4]Department of Microbiology and Immunology, Columbia University Irving Medical Center, New York, NY, USA. [5]Division of Infectious Diseases, Department of Internal Medicine, Columbia University Vagelos College of Physicians and Surgeons, New York, NY, USA. [*]Address correspondence to pw2517@cumc.columbia.edu or dh2994@cumc.columbia.edu

The relative resistance of SARS-CoV-2 variants B.1.1.7 and B.1.351 to antibody neutralization has been described recently. We now report that another emergent variant from Brazil, P.1, is not only refractory to multiple neutralizing monoclonal antibodies, but also more resistant to neutralization by convalescent plasma (6.5 fold) and vaccinee sera (2.2-2.8 fold). The P.1 variant threatens current antibody therapies but less so the protective efficacy of our vaccines.

SARS-CoV-2 P.1, emerging from the B.1.1.28 lineage, has become a dominant variant in Brazil[1,2]. P.1 contains 10 spike mutations[2] in addition to D614G, including K417T, E484K, and N501Y in the receptor-binding domain (RBD), L18F, T20N, P26S, D138Y and R190S in the N-terminal domain (NTD), and H655Y near the furin cleavage site (Supplementary Fig. 1). This new variant could threaten the efficacy of current monoclonal antibody (mAb) therapies or vaccines, because it shares mutations at the same three RBD residues with B.1.351, a variant that first emerged from South Africa. We and others[3-5] have shown that B.1.351 is more resistant to neutralization by some mAbs, convalescent plasma, and vaccinee sera, largely due to a E484K mutation that also exists in P.1. We therefore created, as previously described[3,6,7], a VSV-based SARS-CoV-2 pseudovirus with all 10 mutations of the P.1 variant (BZΔ10) and assessed its susceptibility to neutralization by 18 neutralizing mAbs, 20 convalescent plasma, and 22 vaccinee sera as previously reported[3].

We first assayed the neutralizing activity of four mAbs with emergency use authorization (EUA), including REGN10987 (imdevimab), REGN10933 (casirivimab)[8], LY-CoV555

(bamlanivimab)[9,10], and CB6 (etesevimab)[10,11]. As shown in Fig. 1a (left panel) and Supplementary Fig. 2a, the neutralizing activities of three of the mAbs with EUA were markedly or completely abolished against BZΔ10. The only mAb with EUA retaining its activity was REGN10987. We next tested the neutralizing activity of eight additional RBD mAbs, including ones from our own collection (2-15, 2-7, 1-57, & 2-36)[6] as well as S309[12], COV2-2196 & COV2-2130[13], and C121[14]. The neutralizing activities of the two potent mAbs targeting the receptor-binding motif, 2-15 and C121, were completely lost against BZΔ10 (Fig. 1a, middle panel, and Supplementary Fig. 2a). Other mAbs targeting the "inner side" or the "outer side" of RBD retained their activities against BZΔ10, however. Overall, these findings mimic those observed for B.1.351[3], which should not be surprising since the triple RBD mutations in P.1 and B.1.351 are largely the same.

We also assessed the neutralizing activity of six NTD mAbs[6] against BZΔ10 and WT pseudoviruses (Fig. 1a, right panel; Supplemental Fig. 2b). BZΔ10 was profoundly resistant to neutralization by four NTD antibodies: 2-17, 4-18, 4-19, and 5-7. Interestingly, 5-24 and 4-8, two mAbs targeting the antigenic supersite in NTD[15] that have completely lost neutralizing activity against B.1.351[3], remained active against BZΔ10. To understand the specific mutations responsible for the observed pattern of neutralization, we then tested these NTD mAbs against a panel of pseudoviruses, each containing only a single NTD mutation found in P.1 (Supplementary Fig. 2b). As expected, 5-24 ad 4-8 retained activity against all single-mutation pseudoviruses. P26S only partially accounted for the loss of activity of 4-18; L18F/T20N/D138Y contributed to the loss of activity of 2-17 and 4-19; and L18F/T20N/D138Y/R190S together resulted in the loss of activity of 5-7.

Overall, these neutralization results were consistent with the positions of the P.1 mutations on NTD in relation to the antibody epitopes (Supplemental Fig. 3a). For antibodies 5-24 and 4-8, the mutated residues on NTD were not part of their epitopes (Supplemental Fig. 3b). The drop in neutralization potency of 2-17 is explained by L18F and T20N comprising a part of the epitope, while D138 is proximal to these two residues. However, the loss or activity of 4-18 and 5-7 is not well explained structurally, because their inactivity is likely due to the combined effect of different NTD mutations.

We also examined a panel of convalescent plasma obtained from 20 SARS-CoV-2 patients infected in the Spring of 2020, as previously reported[3]. Each plasma sample was assayed for neutralization against BZΔ10 and WT pseudoviruses. As shown in Supplementary Fig. 4, most (16 of 20) samples lost >2.5-fold neutralizing activity against BZΔ10. The magnitude of the drop in plasma neutralization ID50 titers is summarized in Fig. 1b (left panel), showing a 6.5-fold loss of activity against the variant pseudovirus.

Lastly, 22 vaccinee sera were obtained, as previously reported[3], from 12 individuals who received Moderna SARS-Co-2 mRNA-1273 Vaccine[16] and 10 individuals who received the Pfizer BNT162b2 Covid-19 Vaccine[17]. Each serum sample was assayed for neutralization against BZΔ10 and WT pseudoviruses. The extent of the decline in neutralization activity is summarized in Fig. 1b (middle and right panels), and each neutralization profile is shown in Supplementary Fig. 5. A loss of activity against BZΔ10 was noted for every sample, but the magnitude of the loss was modest (2.8 fold, Moderna;

2.2 fold, Pfizer) and not as striking as was observed against B.1.351 pseudovirus (8.6 fold, Moderna; 6.5 fold, Pfizer).

Overall, the SARS-CoV-2 P.1 variant is of concern because of its rapid rise to dominance as well as its extensive spike mutations, which could lead to antigenic changes detrimental to mAb therapies and vaccine protection. Here we report that P.1 is indeed resistant to neutralization by several RBD-directed mAbs, including three with EUA. The major culprit is the shared E484K mutation, which has emerged independently in over 50 lineages, including in B.1.526 that we[18] and others[19] have identified in New York recently. As for the NTD-directed mAbs, the resistance profiles are markedly different for P.1 and B.1.351, reflecting their distinct sets of mutations in NTD. Both convalescent plasma and vaccinee sera show a significant loss of neutralizing activity against P.1, but the diminution is not as great as that reported against B.1.351[3,20]. Therefore, the threat of increased re-infection or decreased vaccine protection posed by P.1 may not be as severe as B.1.351. Finally, given that the RBD mutations are largely the same for these two variants, the discrepancy in their neutralization susceptibility to polyclonal plasma or sera suggests that NTD mutations can have a significant effect on the susceptibility of SARS-CoV-2 to antibody neutralization.

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Figure

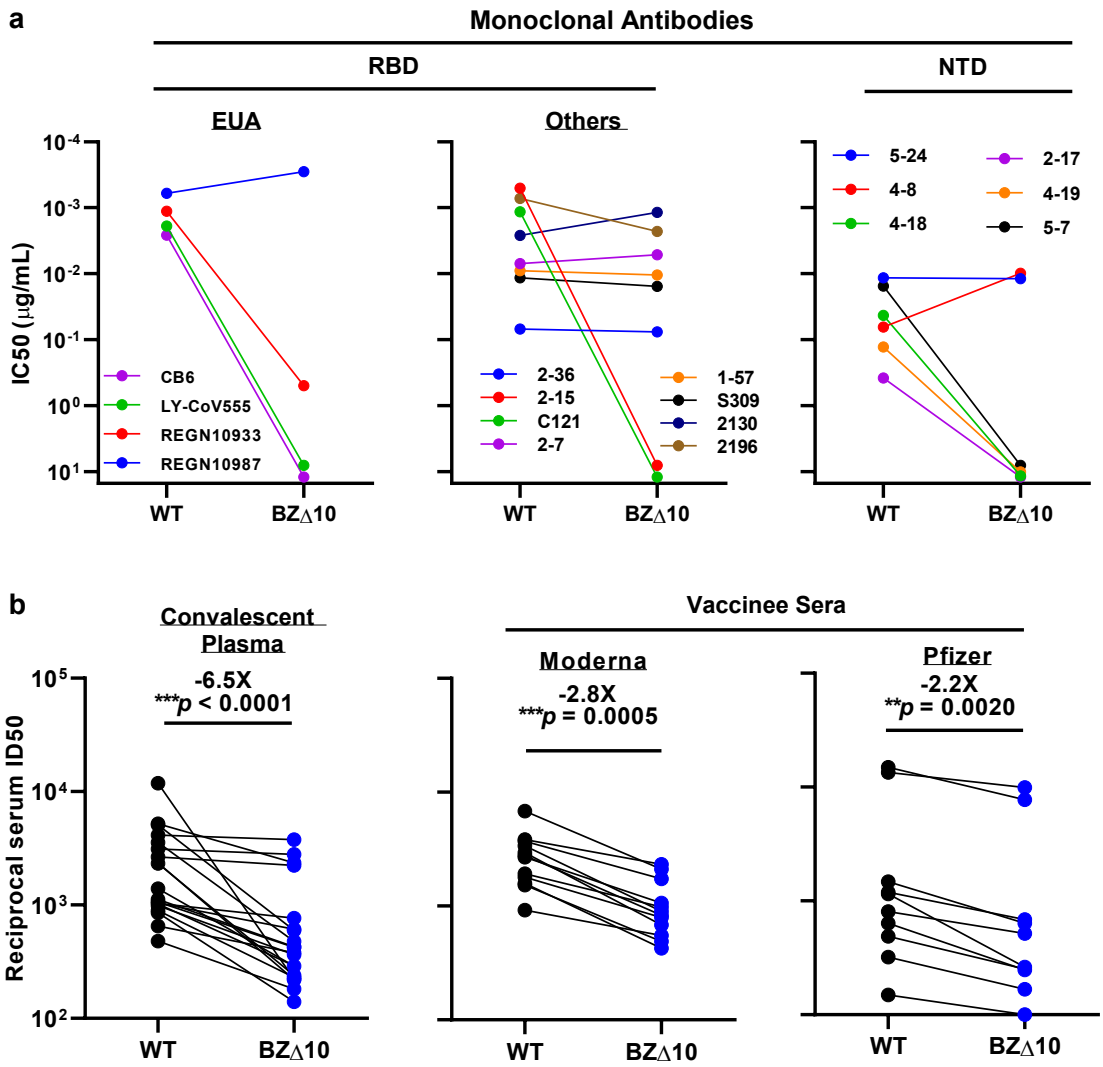

Fig. 1 | Neutralization of WT and BZ△10 pseudoviruses by mAbs, convalescent plasma, and vaccinee sera. a, Changes in neutralization IC50 of select RBD and NTD mAbs. **b,** Changes in reciprocal plasma neutralization ID50 values of convalescent plasma and reciprocal serum ID50 values for persons who received Moderna or Pfizer vaccine. Mean fold change in ID50 relative to the WT is written above the *p* values.

182 Statistical analysis was performed using a Wilcoxon matched-pairs signed rank test. Two-

183 tailed p-values are reported.

Methods

Monoclonal antibodies, patients and vaccinees. Monoclonal antibodies, convalescent plasma, and vaccinee sera were the same as previously reported[3].

Pseudovirus neutralization assays. Plasmids encoding the single-mutation variants found in P.1 and 10-mutation variant (BZΔ10) were generated by Quikchange II XL site-directed mutagenesis kit (Agilent). Recombinant Indiana VSV (rVSV) expressing different SARS-CoV-2 spike variants were generated as previously described[3,6,7]. Neutralization assays were performed by incubating pseudoviruses with serial dilutions of mAbs or heat-inactivated plasma or sera, and scored by the reduction in luciferase gene expression as previously described[3,6,7].

Data availability. Materials used in this study will be made available but may require execution of a material transfer agreement. Source data are provided herein.

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Author contributions. The study was conceptualized by D.D.H. The experiments were principally carried out by P.W., M.W., and J.Y., with assistance from M.S.N., and Y.H. Structural interpretations were made by G.C., L.S., and P.D.K. The manuscript was written by P.W. and D.D.H. and reviewed, commented, and approved by all the authors.

206 **Competing interests:** P.W., J.Y., M.N., Y.H., and D.D.H. are inventors on a provisional

207 patent application on mAbs to SARS-CoV-2.