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

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

38

39 SARS-CoV-2 P.1, emerging from the B.1.1.28 lineage, has become a dominant variant
40 in Brazil^{1,2}. P.1 contains 10 spike mutations² in addition to D614G, including K417T,
41 E484K, and N501Y in the receptor-binding domain (RBD), L18F, T20N, P26S, D138Y
42 and R190S in the N-terminal domain (NTD), and H655Y near the furin cleavage site
43 (Supplementary Fig. 1). This new variant could threaten the efficacy of current
44 monoclonal antibody (mAb) therapies or vaccines, because it shares mutations at the
45 same three RBD residues with B.1.351, a variant that first emerged from South Africa.
46 We and others³⁻⁵ have shown that B.1.351 is more resistant to neutralization by some
47 mAbs, convalescent plasma, and vaccinee sera, largely due to a E484K mutation that
48 also exists in P.1. We therefore created, as previously described^{3,6,7}, a VSV-based
49 SARS-CoV-2 pseudovirus with all 10 mutations of the P.1 variant (BZΔ10) and assessed
50 its susceptibility to neutralization by 18 neutralizing mAbs, 20 convalescent plasma, and
51 22 vaccinee sera as previously reported³.

52

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

55 (bamlanivimab)^{9,10}, and CB6 (etesevimab)^{10,11}. As shown in Fig. 1a (left panel) and
56 Supplementary Fig. 2a, the neutralizing activities of three of the mAbs with EUA were
57 markedly or completely abolished against BZΔ10. The only mAb with EUA retaining its
58 activity was REGN10987. We next tested the neutralizing activity of eight additional RBD
59 mAbs, including ones from our own collection (2-15, 2-7, 1-57, & 2-36)⁶ as well as S309¹²,
60 COV2-2196 & COV2-2130¹³, and C121¹⁴. The neutralizing activities of the two potent
61 mAbs targeting the receptor-binding motif, 2-15 and C121, were completely lost against
62 BZΔ10 (Fig. 1a, middle panel, and Supplementary Fig. 2a). Other mAbs targeting the
63 “inner side” or the “outer side” of RBD retained their activities against BZΔ10, however.
64 Overall, these findings mimic those observed for B.1.351³, which should not be surprising
65 since the triple RBD mutations in P.1 and B.1.351 are largely the same.

66

67 We also assessed the neutralizing activity of six NTD mAbs⁶ against BZΔ10 and WT
68 pseudoviruses (Fig. 1a, right panel; Supplemental Fig. 2b). BZΔ10 was profoundly
69 resistant to neutralization by four NTD antibodies: 2-17, 4-18, 4-19, and 5-7. Interestingly,
70 5-24 and 4-8, two mAbs targeting the antigenic supersite in NTD¹⁵ that have completely
71 lost neutralizing activity against B.1.351³, remained active against BZΔ10. To understand
72 the specific mutations responsible for the observed pattern of neutralization, we then
73 tested these NTD mAbs against a panel of pseudoviruses, each containing only a single
74 NTD mutation found in P.1 (Supplementary Fig. 2b). As expected, 5-24 and 4-8 retained
75 activity against all single-mutation pseudoviruses. P26S only partially accounted for the
76 loss of activity of 4-18; L18F/T20N/D138Y contributed to the loss of activity of 2-17 and
77 4-19; and L18F/T20N/D138Y/R190S together resulted in the loss of activity of 5-7.

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

85
86 We also examined a panel of convalescent plasma obtained from 20 SARS-CoV-2
87 patients infected in the Spring of 2020, as previously reported³. Each plasma sample
88 was assayed for neutralization against BZΔ10 and WT pseudoviruses. As shown in
89 Supplementary Fig. 4, most (16 of 20) samples lost >2.5-fold neutralizing activity against
90 BZΔ10. The magnitude of the drop in plasma neutralization ID50 titers is summarized in
91 Fig. 1b (left panel), showing a 6.5-fold loss of activity against the variant pseudovirus.

92
93 Lastly, 22 vaccinee sera were obtained, as previously reported³, from 12 individuals who
94 received Moderna SARS-Co-2 mRNA-1273 Vaccine¹⁶ and 10 individuals who received
95 the Pfizer BNT162b2 Covid-19 Vaccine¹⁷. Each serum sample was assayed for
96 neutralization against BZΔ10 and WT pseudoviruses. The extent of the decline in
97 neutralization activity is summarized in Fig. 1b (middle and right panels), and each
98 neutralization profile is shown in Supplementary Fig. 5. A loss of activity against BZΔ10
99 was noted for every sample, but the magnitude of the loss was modest (2.8 fold, Moderna;

100 2.2 fold, Pfizer) and not as striking as was observed against B.1.351 pseudovirus (8.6
101 fold, Moderna; 6.5 fold, Pfizer).

102

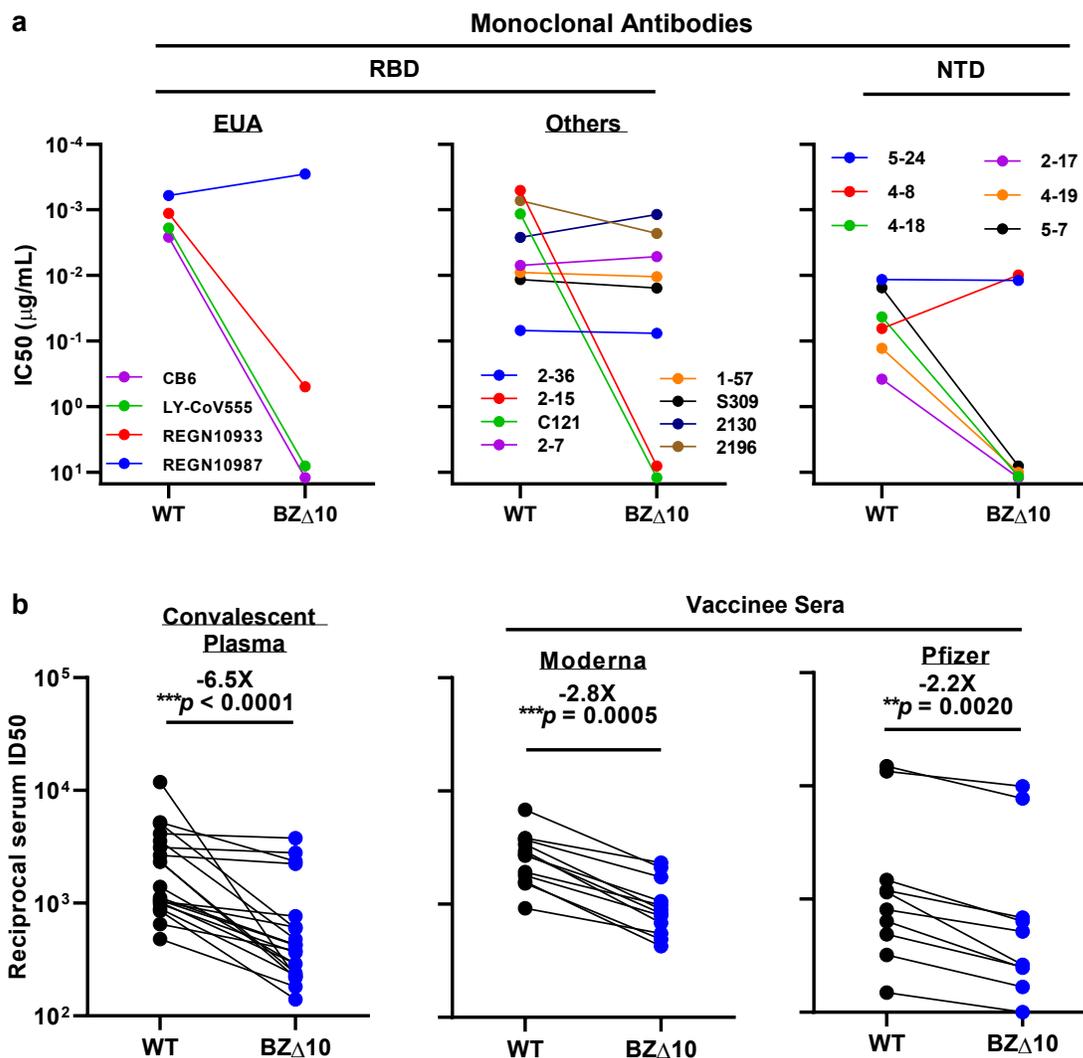
103 Overall, the SARS-CoV-2 P.1 variant is of concern because of its rapid rise to dominance
104 as well as its extensive spike mutations, which could lead to antigenic changes
105 detrimental to mAb therapies and vaccine protection. Here we report that P.1 is indeed
106 resistant to neutralization by several RBD-directed mAbs, including three with EUA. The
107 major culprit is the shared E484K mutation, which has emerged independently in over 50
108 lineages, including in B.1.526 that we¹⁸ and others¹⁹ have identified in New York recently.
109 As for the NTD-directed mAbs, the resistance profiles are markedly different for P.1 and
110 B.1.351, reflecting their distinct sets of mutations in NTD. Both convalescent plasma and
111 vaccinee sera show a significant loss of neutralizing activity against P.1, but the
112 diminution is not as great as that reported against B.1.351^{3,20}. Therefore, the threat of
113 increased re-infection or decreased vaccine protection posed by P.1 may not be as
114 severe as B.1.351. Finally, given that the RBD mutations are largely the same for these
115 two variants, the discrepancy in their neutralization susceptibility to polyclonal plasma or
116 sera suggests that NTD mutations can have a significant effect on the susceptibility of
117 SARS-CoV-2 to antibody neutralization.

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175 **Figure**



176

177 **Fig. 1 | Neutralization of WT and BZ Δ 10 pseudoviruses by mAbs, convalescent**

178 **plasma, and vaccinee sera. a,** Changes in neutralization IC₅₀ of select RBD and NTD

179 **mAbs. b,** Changes in reciprocal plasma neutralization ID₅₀ values of convalescent

180 **plasma and reciprocal serum ID₅₀ values for persons who received Moderna or Pfizer**

181 **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.

184 **Methods**

185 **Monoclonal antibodies, patients and vaccinees.** Monoclonal antibodies, convalescent
186 plasma, and vaccinee sera were the same as previously reported³.

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

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

196
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200

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

205

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.