

Antibody Evasion Properties of SARS-CoV-2 Omicron Sublineages

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22 **Abstract**

23

24 The identification of the Omicron variant (B.1.1.529.1 or BA.1) of SARS-CoV-2 (severe acute
25 respiratory syndrome coronavirus 2) in Botswana in November 2021¹ immediately raised alarms
26 due to the sheer number of mutations in the spike glycoprotein that could lead to striking
27 antibody evasion. We² and others³⁻⁶ recently reported results in this Journal confirming such a
28 concern. Continuing surveillance of Omicron evolution has since revealed the rise in prevalence
29 of two sublineages, BA.1 with an R346K mutation (BA.1+R346K) and B.1.1.529.2 (BA.2), with
30 the latter containing 8 unique spike mutations while lacking 13 spike mutations found in BA.1.
31 We therefore extended our studies to include antigenic characterization of these new sublineages.
32 Polyclonal sera from patients infected by wild-type SARS-CoV-2 or recipients of current mRNA
33 vaccines showed a substantial loss in neutralizing activity against both BA.1+R346K and BA.2,
34 with drops comparable to that already reported for BA.1^{2,3,5,6}. These findings indicate that these
35 three sublineages of Omicron are antigenically equidistant from the wild-type SARS-CoV-2 and
36 thus similarly threaten the efficacies of current vaccines. BA.2 also exhibited marked resistance
37 to 17 of 19 neutralizing monoclonal antibodies tested, including S309 (sotrovimab)⁷, which had
38 retained appreciable activity against BA.1 and BA.1+R346K^{2-4,6}. This new finding shows that
39 no presently approved or authorized monoclonal antibody therapy could adequately cover all
40 sublineages of the Omicron variant.

41

42 **Main Text**

43

44 The meteoric rise of the B.1.1.529/Omicron to become the dominant SARS-CoV-2 variant
45 globally has been truly remarkable⁸. Continuing surveillance of its evolution in the population
46 over the past six weeks has revealed that the proportion of the original form, BA.1, has been
47 decreasing steadily while the proportions of two other sublineages have increased noticeably
48 (**Fig. 1a**). In fact, the BA.1+R346K sublineage now accounts for ~30% of Omicron sequences
49 globally, and ~30-45% in South Africa, United Kingdom, and United States. On the other hand,
50 the BA.2 sublineage accounts for only ~13% of Omicron sequences globally, but it is not only on
51 the rise but also the dominant form in countries such as Denmark and India. These three
52 sublineages of Omicron share 21 mutations in the spike protein, wherein BA.2 contains 8 unique

53 mutations and BA.1 contains 13 unique mutations (**Fig. 1b**). Of course, BA.1+R346K has one
54 mutation more than BA.1. Given these differences, their antigenic properties cannot be assumed
55 to be the same or similar.

56
57 Therefore, we first investigated the neutralization sensitivity of the Omicron sublineages by
58 polyclonal sera from convalescent patients or individuals given mRNA vaccines, with or without
59 a booster shot. These serum samples, as well as the pseudovirus neutralization assay used, were
60 identical to ones previously reported². The wild-type D614G pseudovirus was included as a
61 comparator. As was observed and reported for BA.1^{2,3,5,6}, a marked and significant loss of serum
62 neutralizing activity against BA.1+R346K and BA.2 relative to D614G was noted, with
63 neutralizing titers for numerous samples dropping below the limit of detection (**Fig. 1c**). The loss
64 of neutralizing activity against BA.1+R346K or BA.2 sublineages was less prominent for sera
65 obtained from individuals who received a booster vaccination (**Fig. 1c**, right panel), consistent
66 with reported findings for BA.1^{2,3,6}. Among these samples, the mean serum neutralizing titers
67 against Omicron sublineages were significantly lower than the mean titer for D614G; although
68 the mean titer was slightly lower for BA.2, the difference from BA.1 sublineages did not reach
69 statistical significance ($P = 0.242$).

70
71 To further examine antigenic differences in the spike protein of these Omicron sublineages, a
72 panel of 19 neutralizing monoclonal antibodies was used as probes. Seventeen were directed to
73 different epitope clusters (classes 1-4) within the receptor-binding domain (RBD), whereas two
74 were directed to the N-terminal domain (NTD). These antibodies included REGN10987
75 (imdevimab)⁹, REGN10933 (casirivimab)⁹, COV2-2196 (tixagevimab)¹⁰, COV2-2130
76 (cilgavimab)¹⁰, LY-CoV555 (bamlanivimab)¹¹, CB6 (etesevimab)¹², Brie-196 (amubarvimab)¹³,
77 Brie-198 (romlusevimab)¹³, S309 (sotrovimab)⁷, LY-CoV1404 (bebtelovimab)¹⁴, ADG-2¹⁵,
78 DH1047¹⁶, and S2X259¹⁷, as well as 1-20, 2-15, 2-7, 4-18, 5-7¹⁸ and 10-40¹⁹ from our group.
79 Overall, 17 of 19 monoclonal antibodies were either totally inactive or severely impaired in
80 neutralizing BA.2 (**Fig. 2a**), somewhat like previous findings for BA.1 and BA.1+R346K² but
81 with important differences (**Fig. 2b**). All class 4 antibodies tested lost greater neutralizing
82 potency against BA.2 versus BA.1 sublineages. Two class 3 antibodies, COV2-2130 and 2-7,
83 retained decent activity against BA.2 while having no activity against BA.1 viruses. S309 or

84 sotrovimab lost 27-fold neutralizing activity against BA.2; this is particularly important because
85 it was found to be the only clinically approved or authorized monoclonal antibody to retain
86 activity against the original form of Omicron²⁻⁴. LY-CoV1404, another class 3 antibody in
87 development, remained potent in neutralizing all Omicron sublineages, suggesting that there is
88 still a patch within this antibody-binding region that is unaffected by all spike mutations found in
89 SARS-CoV-2 variants to date. Although there was a lack of an observable difference among the
90 Omicron sublineages in neutralization by polyclonal sera (**Fig. 1c**), important antigenic
91 differences do exist when probed by monoclonal antibodies. Except for S309, BA.1 appears to
92 be more resistant to class 3 antibodies than BA.2, while BA.2 is more resistant to all class 4
93 antibodies tested. Our recent study² showed that previous SARS-CoV-2 variants, such as
94 B.1.351/Beta and B.1.617.2/Delta, evolved to resist class 1, class 2, and NTD antibodies first,
95 and then the Omicron variant seemingly has further evolved to resist class 3 and class 4
96 antibodies in addition. Our current findings suggest that the Omicron sublineages may have
97 diverged under slightly different pressure from class 3 and class 4 antibodies to the RBD.

98
99 Finally, we constructed each of the eight BA.2-specific spike mutations alone as pseudoviruses
100 and tested them using the same panel of 19 monoclonal antibodies (**Fig. 2b**). S371F broadly
101 affected most of the RBD-directed antibodies, similar to what was observed for S371L in BA.1²
102 but with a greater negative impact, perhaps due to the bulkier side chain of phenylalanine.
103 Intriguingly but importantly, S371F appears to be majorly responsible for the loss in potency of
104 S309, although this mutation was not observed previously as a marker for clinical resistance to
105 sotrovimab²⁰. CB6 was adversely affected by the D405N mutation, likely due to its position
106 within the epitope of this antibody¹². It is not clear how T19I and L24S mutations in the NTD
107 subtly impaired the neutralizing activity of class 1 antibodies to RBD.

108
109 In summary, we have comprehensively evaluated the antigenic properties of two sublineages of
110 the Omicron variant, BA.1+R346K and BA.2, and we believe our results have important clinical
111 implications. First, polyclonal sera showed a substantial loss in neutralizing activity against both
112 sublineages, with drops comparable to that of BA.1 (**Fig. 1c**). These three sublineages of
113 Omicron, therefore, seem to be antigenically equidistant from the wild-type SARS-CoV-2, likely
114 threatening the efficacies of current COVID-19 vaccines to a similar extent. The present study,

115 however, does not address the antigenic distance between BA.1 and BA.2, which will require
116 cross-neutralization experiments using sublineage-specific sera to determine. Second,
117 monoclonal antibodies were affected in a disparate manner for the different Omicron sublineages.
118 For clinically approved or authorized antibodies, only S309 (sotrovimab) retained activity
119 against both BA.1 and BA.1+R346K, but its activity against BA.2 has dropped 27-fold (**Fig. 2b**)
120 to a 50% inhibitory concentration (IC₅₀) of ~1 µg/mL (**Fig. 2a**). Only COV2-2130 (cilgavimab)
121 and its combination with COV2-2196 (tixagevimab) retained activity against BA.2, but this
122 antibody combination is only authorized for preventive use. Presently, no authorized therapeutic
123 monoclonal antibody could adequately treat all sublineages of the Omicron variant. This finding
124 poses a therapeutic dilemma in geographic regions where all three sublineages are present in
125 sufficient numbers. As COVID-19 treatment options are narrowed by the emergence of more and
126 more variants, it is imperative that we continue to devise novel strategies to contain this ever-
127 evolving pathogen.

128 **Figure Legends**

129

130 **Fig. 1 | BA.2 exhibits a similar serum neutralization profile as BA.1 sublineages. a,**
131 Proportions of BA.1, BA.1+R346K, and BA.2 within B.1.1.529 sequences on GISAID over the
132 past six weeks. Values in the upper right corner of each box denote cumulative number of
133 Omicron sequences. **b,** Mutations within the B.1.1.529 lineage. **c,** Pseudovirus neutralization by
134 convalescent and vaccinee sera. Values above points indicate the geometric mean. Numbers in
135 parentheses denote the number of samples above the limit of detection (LOD) of 100. Values
136 below the LOD are arbitrarily plotted to allow for visualization of each sample. P values were
137 determined by two-sided Friedman test followed by Dunn's multiple comparisons test.

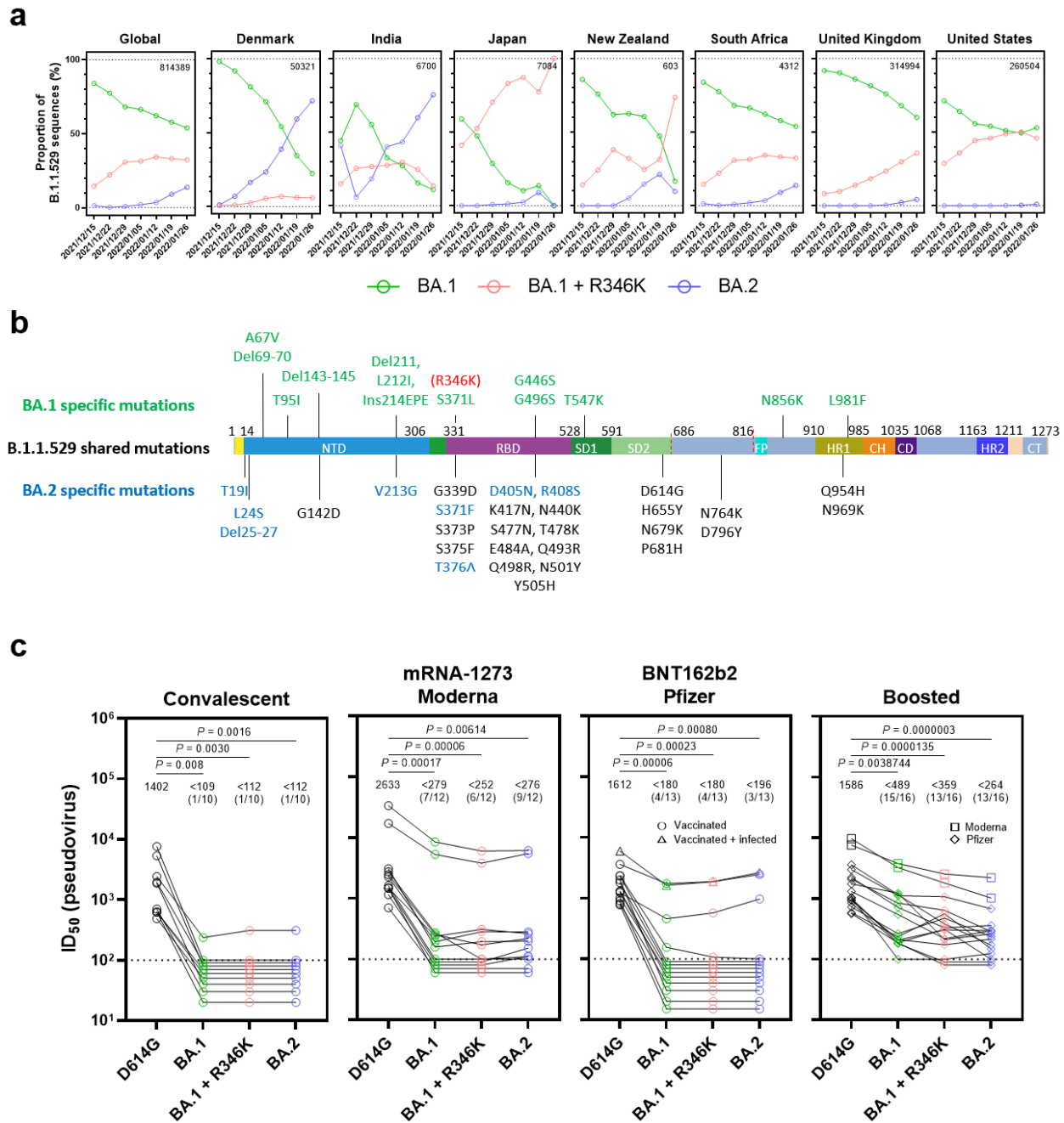
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139 **Fig. 2 | BA.2 differs in resistance profile to monoclonal antibodies. a,** Pseudovirus
140 neutralization by monoclonal antibodies. Values above the LOD of 10 $\mu\text{g/mL}$ are arbitrarily
141 plotted to allow for visualization of each sample. **b,** Fold change in IC_{50} values relative to D614G
142 of neutralization of Omicron variants, as well as point mutants unique to BA.2.

143

144 **Figure 1**

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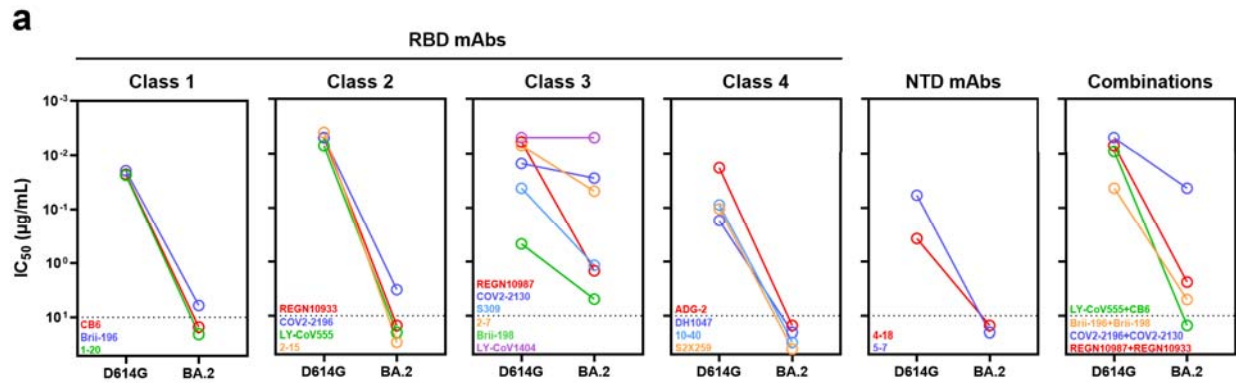


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147

148 **Figure 2**

149



b

Fold change in IC ₅₀ relative to D614G	RBD mAbs															NTD mAbs			
	Class 1			Class 2				Class 3					Class 4			4-18	5-7		
	CB6	Brij-196	1-20	REGN 10933	COV2-2196	LY-CoV 555	2-15	REGN 10987	COV2-2130	S309	2-7	Brij-196	LY-CoV 1404	ADG-2	DH1047			10-40	S2X259
BA.1	<-428	-298	<-429	<-2201	-306	<-1496	<-2716	<-1716	-83.5	-6.9	-195	2.3	1.4	-11.0	-14.2	-21.1	-13.7	<-26.7	-4.1
BA.1 + R346K	<-428	-135	<-429	-415	-187	<-1496	<-2716	<-1716	<-687	-4.5	-82.1	<-22	1.5	-15.7	-7.9	-20.5	-7.5	<-26.7	-5.5
BA.2	<-428	-322	<-429	<-2201	-680	<-1496	<-2716	-253	-1.9	-27.0	-7.3	-10.5	1.1	<-555	<-58.0	<-114	<-96	<-26.7	<-171
T19I	-3.1	-4.9	-5.3	-3.7	-1.9	-2.2	-2.0	-2.1	-1.5	-1.8	-5.1	-1.6	-1.7	-1.7	-1.5	-2.7	-2.9	-6.1	-3.3
L24S	-2.9	-4.0	-4.6	-3.2	-2.4	-2.4	-2.8	-4.2	-2.1	-1.5	-2.6	-2.2	-1.6	-1.3	-1.1	-2.4	-2.0	-3.1	-1.1
Del25-27	-1.2	-2.6	-2.0	-1.3	-1.0	-1.4	-1.2	-1.3	1.0	-1.3	-2.8	2.0	-1.2	1.1	1.6	-1.8	1.1	-23.1	-16.8
V213G	-2.5	-3.1	-3.0	-3.1	-1.5	-1.1	-1.6	-2.2	-2.0	-1.2	-3.2	-1.1	-1.5	1.1	1.0	-2.0	-1.7	1.9	-2.8
S371F	-143	-126	-95.1	-27.9	-26.1	-5.1	-6.3	-86.6	-1.3	-20.5	-30.6	<-22	-2.4	-43.0	-60.9	<-114	-77.5	7.8	2.3
T376A	-1.9	-3.1	-2.5	-2.1	-1.3	-1.7	-1.3	-1.9	-1.8	1.0	-2.7	2.0	-1.7	1.1	1.1	-1.5	-2.3	1.3	-1.3
D405N	-25.6	-2.3	-2.9	-2.8	-2.1	-1.9	-1.7	-1.6	1.0	1.5	-3.1	-1.6	1.3	3.3	-1.2	-3.9	-2.2	5.6	1.5
R408S	1.4	-1.1	-1.3	-1.1	1.5	-1.6	-1.3	1.2	1.0	1.0	1.2	1.4	-1.4	-1.6	-2.1	-1.2	-3.6	1.1	-1.3

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>3 <-3 <-10 <-100

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152

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197

198 **Methods**

199

200 **Data reporting**

201 No statistical methods were used to predetermine sample size. The experiments were not
202 randomized and the investigators were not blinded to allocation during experiments and outcome
203 assessment.

204

205 **Serum samples**

206 Identical samples from a previous study were utilized². All collections were conducted under
207 protocols reviewed and approved by the Institutional Review Board of Columbia University.

208

209 **Antibodies and pseudovirus neutralization**

210 The expression of antibodies, construction of variant SARS-CoV-2 spike plasmids, production
211 and neutralization of pseudoviruses, were conducted as previously described².

212

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218

219 **Author contributions**

220 D.D.H. conceived this project. S.I. and Lihong Liu conducted pseudovirus neutralization
221 experiments. Y.G. and Z.Z. conducted bioinformatic analyses. Liyuan Liu and Yiming Huang
222 constructed the spike expression plasmids. M.W. aided sample collections. Y.L. managed the
223 project. J.Y. expressed and purified antibodies. M.T.Y. and M.E.S. provided clinical samples.
224 Yaoxing Huang contributed to discussions. H.H.W. and D.D.H. directed and supervised the
225 project. S.I, Lihong Liu, and D.D.H. analyzed the results and wrote the manuscript.

226

227 **Competing interests**

228 S.I, Lihong Liu, J.Y., Yaoxing Huang, and D.D.H. are inventors on patent applications
229 (WO2021236998) or provisional patent applications (63/271,627) filed by Columbia University
230 for a number of SARS-CoV-2 neutralizing antibodies described in this manuscript. Both sets of
231 applications are under review. D.D.H. is a co-founder of TaiMed Biologics and RenBio,
232 consultant to WuXi Biologics and Bii Biosciences, and board director for Vicarious Surgical.

233

234 **Data and materials availability**

235 All data are provided in the manuscript.

236