

1 **Neutralization of SARS-CoV-2 spike 69/70 deletion, E484K, and N501Y variants by**
2 **BNT162b2 vaccine-elicited sera**

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19

20 **Abstract**

21 We engineered three SARS-CoV-2 viruses containing key spike mutations from the
22 newly emerged United Kingdom (UK) and South African (SA) variants: N501Y from UK and SA;
23 69/70-deletion+N501Y+D614G from UK; and E484K+N501Y+D614G from SA. Neutralization
24 geometric mean titers (GMTs) of twenty BNT162b2 vaccine-elicited human sera against the
25 three mutant viruses were 0.81- to 1.46-fold of the GMTs against parental virus, indicating small
26 effects of these mutations on neutralization by sera elicited by two BNT162b2 doses.

27

28 **Main**

29 We previously reported that BNT162b2, a nucleoside modified RNA vaccine that
30 encodes the SARS-CoV-2 full length, prefusion stabilized spike glycoprotein (S), elicited dose-
31 dependent SARS-CoV-2-neutralizing geometric mean titers (GMTs) that were similar to or
32 higher than the GMT of a panel of SARS-CoV-2 convalescent human serum samples.¹ We
33 subsequently reported that, in a randomized, placebo-controlled trial in approximately 44,000
34 participants 16 years of age or older, a two-dose regimen of BNT162b2 conferred 95%
35 protection against COVID-19.²

36 Since the previously reported studies were conducted, rapidly spreading variants of
37 SARS-CoV-2 have arisen in the United Kingdom (UK), South Africa (SA), and other regions.^{3,4}
38 These variants have multiple mutations in their spike glycoproteins, which are key targets of
39 virus neutralizing antibodies. The emerged spike mutations have raised concerns of vaccine
40 efficacy against these new strains. The goal of this study is to examine the effect of several key
41 spike mutations from the UK and SA strains on BNT162b2 vaccine-elicited neutralization.

42 Using an infectious cDNA clone of SARS-CoV-2⁵, we engineered three spike mutant
43 viruses on the genetic background of clinical strain USA-WA1/2020 (**Supplementary Fig. 1**). (i)
44 Mutant N501Y virus contains the N501Y mutation that is shared by both the UK and SA variants.
45 This mutation is located in the viral receptor binding domain (RBD) for cell entry, increases
46 binding to the angiotensin converting enzyme 2 (ACE2) receptor, and enables the virus to
47 expand its host range to infect mice.^{5,6} (ii) Mutant $\Delta 69/70$ +N501Y+D614G virus contains two
48 additional changes present in the UK variants: amino acid 69 and 70 deletion ($\Delta 69/70$) and
49 D614G substitution. Amino acids 69 and 70 are located in the N-terminal domain of the spike S1
50 fragment; deletion of these residues may allosterically change S1 conformation.⁶ The D614G
51 mutation is dominant in circulating strains around the world.^{7,8} (iii) Mutant
52 E484K+N501Y+D614G virus additionally contains the E484K substitution, which is also located

53 in the viral RBD. The E484K substitution alone confers resistance to several monoclonal
54 antibodies.^{9,10} Compared with the wild-type USA-WA1/2020 strain, the three mutant viruses
55 showed similar plaque morphologies on Vero E6 cells (**Supplementary Fig. 2**).

56 We tested a panel of human sera from twenty participants in the previously reported
57 clinical trial,^{1,2} drawn 2 or 4 weeks after immunization with two 30- μ g doses of BNT162b2
58 spaced three weeks apart (**Supplementary Fig. 3**). All neutralization assays were done with the
59 same 20 sera samples, with the two experiments (as described in **Fig. 1** legend) done at
60 different times. Each serum was tested for neutralization of wild-type USA-WA1/2020 strain and
61 the three mutant viruses by a 50% plaque reduction neutralization assay (PRNT₅₀;
62 **Supplementary Tables 1 and 2**). All sera showed equivalent neutralization titers between the
63 wild-type and mutant viruses, with differences of ≤ 4 fold (**Fig. 1**). Notably, ten out of the twenty
64 sera had neutralization titers against mutant $\Delta 69/70+N501Y+D614G$ virus that were twice their
65 titers against the wild-type virus (**Fig. 1b**), whereas six out of the twenty sera had neutralization
66 titers against mutant E484K+N501Y+D614G virus that were half their titers against the wild-type
67 virus (**Fig. 1c**). The ratios of the neutralization GMTs of the sera against the N501Y,
68 $\Delta 69/70+N501Y+D614G$, and E484K+N501Y+D614G viruses to their GMTs against the USA-
69 WA1/2020 virus were 1.46, 1.41, and 0.81, respectively (**Supplementary Fig. 4**).

70 Consistent with other recent reports of the neutralization of SARS-CoV-2 variants or
71 corresponding pseudoviruses by convalescent or post-immunization sera,^{11,12} the neutralization
72 GMT of the serum panel against the virus with three mutations from the SA variant
73 (E484K+N501Y+D614G) was slightly lower than the neutralization GMTs against the N501Y
74 virus or the virus with three mutations from the UK variant ($\Delta 69/70+N501Y+D614G$). However,
75 the magnitude of the differences in neutralization GMTs against any of the mutant viruses in this
76 study was small (0.81- to 1.41-fold), as compared to the 4-fold differences in hemagglutination-

77 inhibition titers that have been used to signal potential need for a strain change in influenza
78 vaccines.¹³

79 A limitation of the current study is that the engineered viruses do not include the full set
80 of spike mutations found in the UK or SA variants.^{3,4} Nevertheless, preserved neutralization of
81 N501Y, $\Delta 69/70$ +N501Y+D614G, and E484K+N501Y+D614G viruses by BNT162b2 vaccine-
82 elicited human sera is consistent with preserved neutralization of a panel of 15 pseudoviruses
83 bearing spikes with other single mutations found in circulating SARS-CoV-2 strains.¹⁴ The
84 emergence of the common mutation N501Y from different geographical regions, as well as the
85 previously emerged globally dominant D614G mutation, suggest that these mutations may
86 improve viral fitness, as recently demonstrated for the increased viral transmission by the
87 D614G mutation in animal models.^{7,15} The biological functions of N501Y and the other mutations
88 (such as $\Delta 69/70$ and E484K) remain to be defined for viral replication, pathogenesis, and/or
89 transmission in animal models. A second limitation of the study is that no serological correlate of
90 protection against COVID-19 has been defined. Therefore, predictions about vaccine efficacy
91 based on neutralization titers require assumptions about the levels of neutralization and roles of
92 humoral and cell-mediated immunity in vaccine-mediated protection. Clinical data are needed
93 for firm conclusions about vaccine effectiveness against variant viruses.

94 The ongoing evolution of SARS-CoV-2 necessitates continuous monitoring of the
95 significance of changes for vaccine efficacy. This surveillance should be accompanied by
96 preparations for the possibility that future mutations may necessitate changes to vaccine strains.
97 The serological criteria for strain changes of influenza vaccine have been well-accepted.¹⁶ For
98 COVID-19, such vaccine updates would be facilitated by the flexibility of mRNA-based vaccine
99 technology.

100

101 **Methods**

102 **Construction of isogenic viruses.** Three recombinant SARS-CoV-2 mutants (N501Y,
103 Δ69/70-N501Y+D614G, E484K+N501Y+D614G in spike protein) were prepared on the genetic
104 background of an infectious cDNA clone derived from clinical strain WA1 (2019-
105 nCoV/USA_WA1/2020)⁵ by following the PCR-based mutagenesis protocol as reported
106 previously⁷. The full-length infectious cDNAs were *in vitro* ligated and used as templates to
107 transcribe full-length viral RNA. Mutant viruses (P0) were recovered on day 2 from Vero E6 cells
108 after electroporation of the *in vitro* RNA transcripts. P1 viruses were harvested as stocks by
109 passaging the P0 virus once on Vero E6 cells. The titers of P1 viruses were determined by
110 plaque assay on Vero E6 cells. The genome sequences of the P1 viruses were validated by
111 Sanger sequencing. The detailed protocol was recently reported¹⁷.

112 **Serum specimens and neutralization assay.** Serum samples were collected from
113 BNT162b2 vaccinees participating in the phase 1 portion of the ongoing phase 1/2/3 clinical trial
114 (ClinicalTrials.gov identifier: NCT04368728). The protocol and informed consent were approved
115 by institutional review boards for each of the investigational centers participating in the study.
116 The study was conducted in compliance with all International Council for Harmonisation (ICH)
117 Good Clinical Practice (GCP) guidelines and the ethical principles of the Declaration of Helsinki.

118 The immunization and serum collection regimen are illustrated schematically in **Fig. S3**.
119 A conventional (non-fluorescent) plaque reduction neutralization assay was performed to
120 quantify the serum-mediated virus suppression as previously reported¹⁸. Briefly, each serum
121 was 2-fold serially diluted in culture medium with the first dilution of 1:40 (dilution range of 1:40
122 to 1:1280). The diluted sera were incubated with 100 plaque-forming units of wild-type or mutant
123 viruses at 37°C for 1 h, after which the serum-virus mixtures were inoculated onto Vero E6 cell
124 monolayer in 6-well plates. After 1 h of infection at 37°C, 2 ml of 2% Seaplaque agar (Lonza) in
125 Dulbecco's modified Eagle medium (DMEM) containing 2% fetal bovine serum (FBS) and 1%
126 penicillin/streptomycin (P/S) was added to the cells. After 2 days of incubation, 2 ml of 2%

127 Seaplaque agar (Lonza) in DMEM containing 2% FBS, 1% P/S and 0.01% neutral red (Sigma)
128 were added on top of the first layer. After another 16 h of incubation at 37°C, plaque numbers
129 were counted. The minimal serum dilution that inhibits 50% of plaque counts is defined as the
130 50% plaque reduction neutralization titer (PRNT₅₀). Each serum was tested in duplicates. The
131 PRNT₅₀ assay was performed at the biosafety level-3 facility with the approval from the
132 Institutional Biosafety Committee at the University of Texas Medical Branch.

133 **Statistics.** No statistics were performed in the study.

134

135 **Data availability**

136 The data that support the findings of this study are available from the corresponding
137 authors upon request.

138

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180

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190

191 **Author contributions**

192 Conceptualization, X.X., V.D.M., S.W., P.-Y.S.; Methodology, X.X., Y.L., J.L., J.Z.,
193 C.R.F.G., H.X., P.-Y.S; Investigation, X.X., Y.L., J.L., J.Z., C.R.F.G., H.X., K.A.S., D.C., P.R.D.,

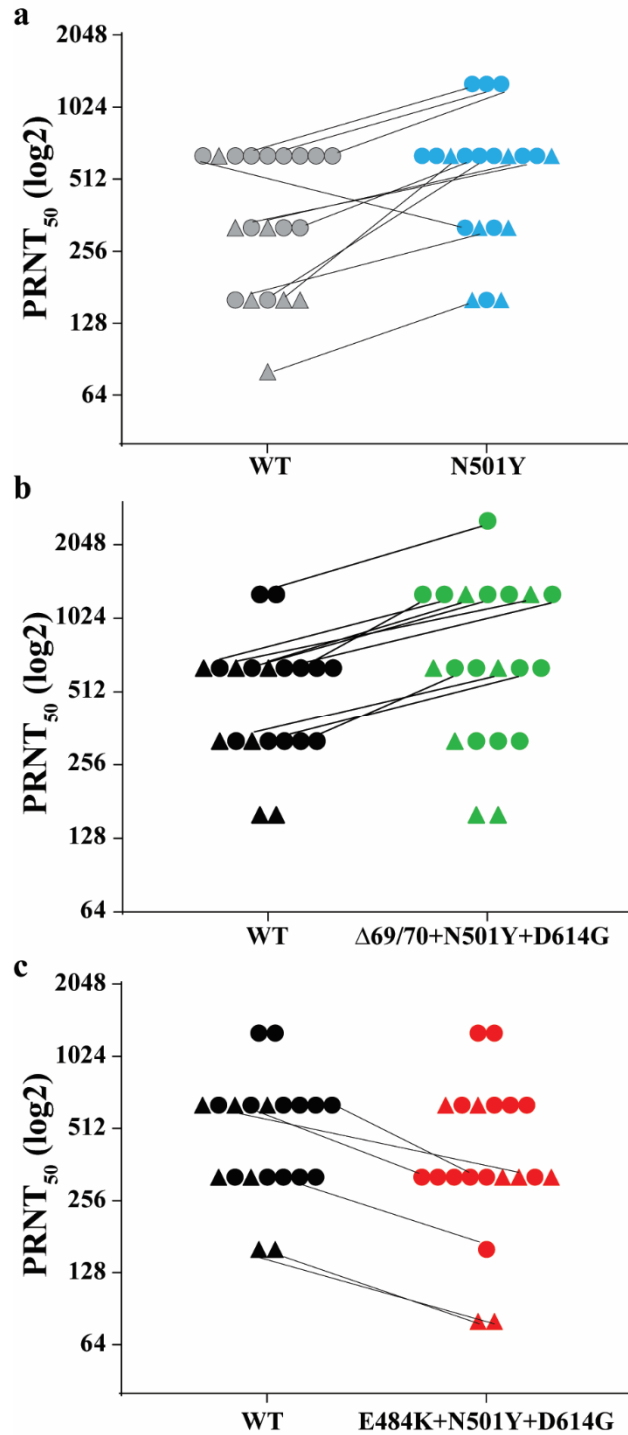
194 P.-Y.S; Resources, M.C., D.C., P.R.D., P.-Y.S; Data Curation, X.X., Y.L., J.L., J.Z., C.R.F.G.,
195 P.-Y.S; Writing-Original Draft, X.X., P.-Y.S; Writing-Review & Editing, X.X., P.R.D., P.-Y.S.;
196 Supervision, X.X., M.C., D.C., P.R.D., P.-Y.S.; Funding Acquisition P.-Y.S.

197

198 **Ethics declarations**

199 Competing interests

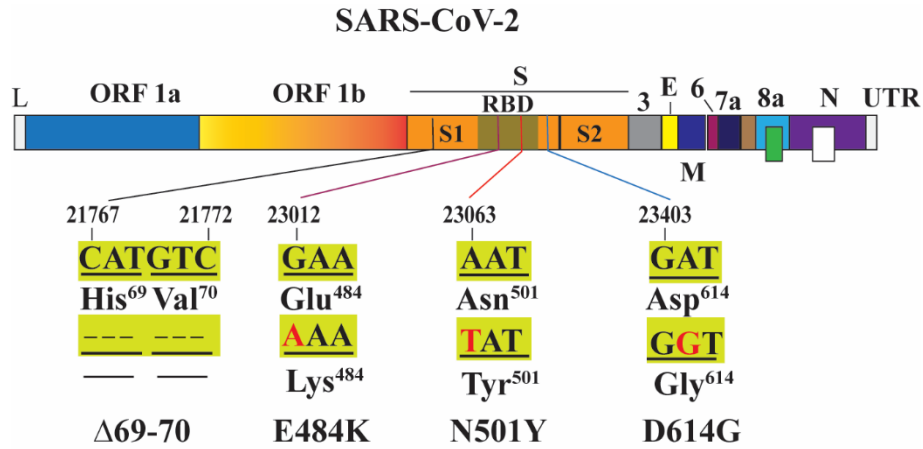
200 X.X., V.D.M., and P.-Y.S. have filed a patent on the reverse genetic system. K.A.S.,
201 M.C., D.C., and P.R.D. are employees of Pfizer and may hold stock options. X.X., J.Z., C.R.F.G.,
202 H.X., and P.-Y.S. received compensation from Pfizer to perform the neutralization assay. Other
203 authors declare no competing interests.



204

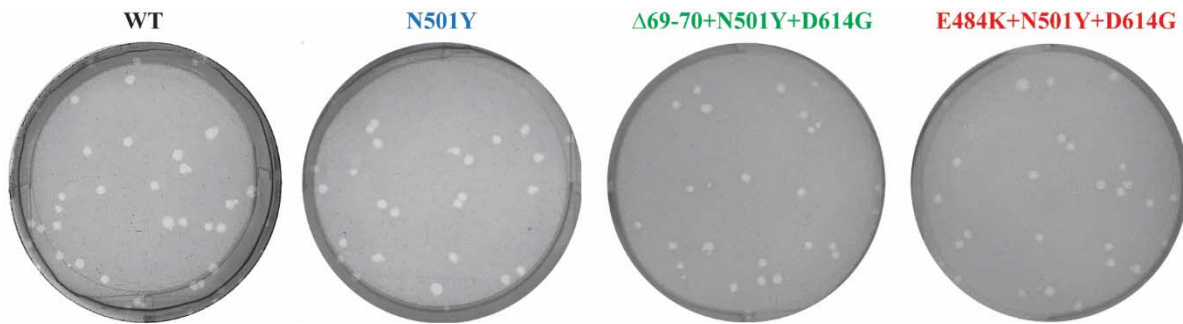
205 **Figure 1. PRNT₅₀s of twenty BNT162b2-vaccinated human sera against wild-type (WT)**
206 **and mutant SARS-CoV-2. (a) WT (USA-WA1/2020) and mutant N501Y. (b) WT and**
207 **Δ69/70+N501Y+D614G. (c) WT and E484K+N501Y+D614G. Seven (triangles) and thirteen**
208 **(circles) sera were drawn 2 and 4 weeks after the second dose of vaccination, respectively.**
209 **Sera with different PRNT₅₀s against WT and mutant viruses are connected by lines. Results in**
210 **(a) were from one experiment; results in (b) and (c) were from another set of experiments. Each**
211 **data point is the average of duplicate assay results.**

212 **Supplementary information**



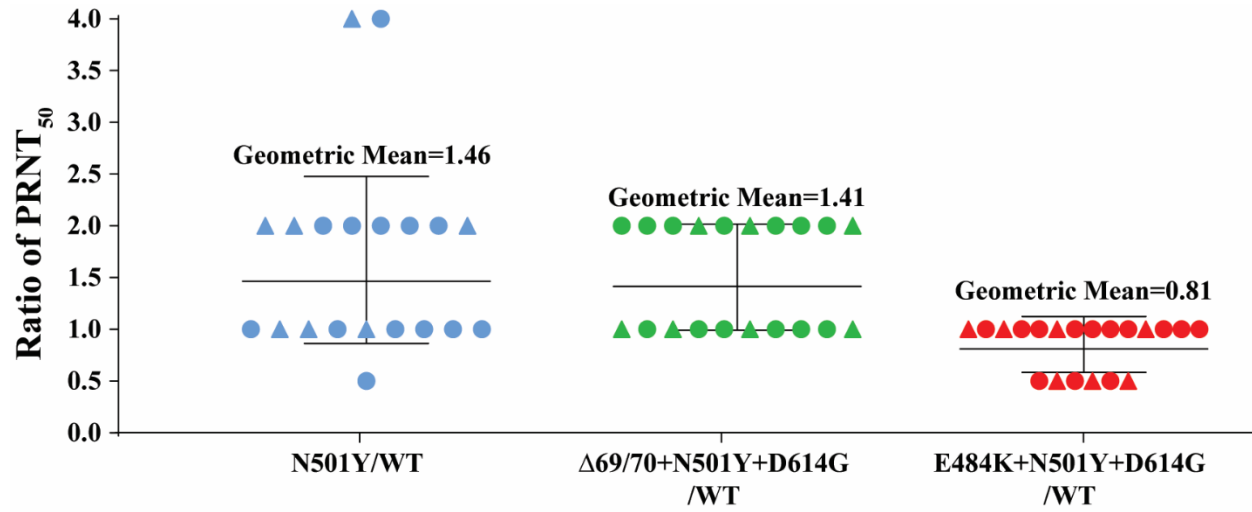
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214 **Supplementary Figure 1. Engineered mutations.** Nucleotide and amino acid positions are
 215 indicated. Deletions are depicted by dotted lines. Mutant nucleotides are in red. L, leader
 216 sequence; ORF, open reading frame; RBD, receptor binding domain; S, spike glycoprotein; S1,
 217 N-terminal furin cleavage fragment of S; S2, C-terminal furin cleavage fragment of S; E,
 218 envelope protein; M, membrane protein; N, nucleoprotein; UTR, untranslated region.



219

220 **Supplementary Figure 2. Plaque morphologies of WT (USA-WA1/2020), mutant N501Y,**
221 **Δ69/70+N501Y+D614G, and E484K+N501Y+D614G SARS-CoV-2s on Vero E6 cells.**



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Supplementary Figure 4. Ratios of neutralization GMTs against mutant viruses to GMTs against WT virus. Triangles represent sera drawn two weeks after the second dose of vaccination; circles represent sera drawn four weeks after the second dose of vaccination.

228 **Supplementary Table 1. PRNT₅₀s of twenty BNT162b2 post-immunization sera against**
229 **wild-type (USA-WA1/2020) and mutant N501Y SARS-CoV-2s**

Serum ID	PRNT ₅₀		PRNT ₅₀ ratio (N501Y/WT)
	WT	N501Y	
1	160	640	4
2	160	320	2
3	320	640	2
4	80	160	2
5	160	160	1
6	320	320	1
7	640	640	1
8	160	160	1
9	640	640	1
10	640	1280	2
11	160	640	4
12	320	320	1
13	640	1280	2
14	640	320	0.5
15	320	640	2
16	320	640	2
17	640	640	1
18	640	1280	2
19	640	640	1
20	640	640	1

230

231

232 **Supplementary Table 2. PRNT₅₀s of twenty BNT162b2 post-immunization sera against**
 233 **wild-type (USA-WA1/2020), Δ69/70+N501Y+D614G, and E484K+N501Y+D614G SARS-CoV-**
 234 **2s**

Seru m ID	PRNT ₅₀			PRNT ₅₀ ratio	
	WT	Δ69/70+N501 Y+D614G	E484K+N501Y+ D614G	Δ69/70+N501Y+ D614G/WT	E484K+N501Y+ D614G/WT
1	320	640	320	2	1
2	160	160	80	1	0.5
3	640	1280	640	2	1
4	160	160	80	1	0.5
5	320	320	320	1	1
6	640	640	640	1	1
7	640	1280	320	2	0.5
8	320	320	160	1	0.5
9	1280	1280	1280	1	1
10	640	1280	640	2	1
11	320	320	320	1	1
12	640	1280	320	2	0.5
13	1280	2560	1280	2	1
14	320	320	320	1	1
15	320	640	320	2	1
16	640	640	640	1	1
17	640	1280	640	2	1
18	320	640	320	2	1
19	640	640	320	1	0.5
20	640	1280	640	2	1

235