

1 mRNA bivalent booster enhances neutralization against
2 BA.2.75.2 and BQ.1.1

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20 **Abstract**

21 The emergence of the highly divergent SARS-CoV-2 Omicron variant has jeopardized the
22 efficacy of vaccines based on the ancestral spike. The bivalent COVID-19 mRNA booster
23 vaccine within the United States is comprised of the ancestral and the Omicron BA.5 spike.
24 Since its approval and distribution, additional Omicron subvariants have been identified with key
25 mutations within the spike protein receptor binding domain that are predicted to escape vaccine
26 sera. Of particular concern is the R346T mutation which has arisen in multiple subvariants,
27 including BA.2.75.2 and BQ.1.1. Using a live virus neutralization assay, we evaluated serum
28 samples from individuals who had received either one or two monovalent boosters or the
29 bivalent booster to determine neutralizing activity against wild-type (WA1/2020) virus and
30 Omicron subvariants BA.1, BA.5, BA.2.75.2, and BQ.1.1. In the one monovalent booster cohort,
31 relative to WA1/2020, we observed a reduction in neutralization titers of 9-15-fold against BA.1
32 and BA.5 and 28-39-fold against BA.2.75.2 and BQ.1.1. In the BA.5-containing bivalent booster
33 cohort, the neutralizing activity improved against all the Omicron subvariants. Relative to
34 WA1/2020, we observed a reduction in neutralization titers of 3.7- and 4-fold against BA.1 and
35 BA.5, respectively, and 11.5- and 21-fold against BA.2.75.2 and BQ.1.1, respectively. These
36 data suggest that the bivalent mRNA booster vaccine broadens humoral immunity against the
37 Omicron subvariants.

38 Results

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40 The emergence of the highly divergent Omicron variant of SARS-CoV-2 led to concerns about
41 the efficacy of vaccines based on the ancestral spike, and the approval of bivalent COVID-19
42 vaccines within the United States (the ancestral spike and the Omicron subvariant BA.5 spike
43 proteins)¹⁻⁴. Since its approval and distribution, additional subvariants have been identified with
44 key mutations that further escape vaccine-elicited antibodies and approved monoclonal
45 antibodies⁵. Of particular concern is the R346T mutation which has arisen in multiple variants of
46 different lineages, including BA.2.75.2 and BQ.1.1 (**Supplementary Fig. 1**). We tested serum
47 samples from individuals who had received either one or two monovalent boosters or the
48 bivalent booster to determine neutralization efficiency against wild-type (WA1/2020) virus and
49 Omicron subvariants BA.1, BA.5, BA.2.75.2, and BQ.1.1 using a live virus neutralization assay.

50 We used an *in vitro*, live-virus focus neutralization test (FRNT) assay in a VeroE6-TMPRSS2
51 cell line¹ to compare the neutralizing activity of serum from individuals who received one
52 monovalent booster (7-28 days after vaccination), two monovalent boosters (70-100 days after
53 vaccination), or the bivalent booster (16-42 days after vaccination). The fold change in
54 neutralizing antibody response among these three cohorts were quantitated by comparing the
55 FRNT₅₀ GMT (geometric mean titer) values of Omicron against the ancestral SARS-CoV-2
56 virus. Samples that fell below the limit of detection (1:20) were given an arbitrary FRNT₅₀ of 10.

57 In all groups, a decrease in neutralization activity was observed against all omicron subvariants
58 compared to WA1/2020, with the greatest decrease seen against BQ.1.1 (**Fig. 1**). In the one
59 monovalent booster cohort, the FRNT₅₀ GMTs were 758 for WA1/2020, 60 for BA.1, 50 for
60 BA.5, 23 for BA.2.75.2 and 19 for BQ.1.1. In the two monovalent booster cohort, the FRNT₅₀
61 GMTs were 1812 for WA1/2020, 205 for BA.1, 142 for BA.5, 65 for BA.2.75.2 and 53 for BQ.1.1.
62 In both cohorts, relative to WA1/2020, this corresponded to a reduction in neutralization titers of
63 9-15 fold against BA.1 and BA.5 and 28-39 fold against BA.2.75.2 and BQ.1.1. BA.2.75 showed
64 comparable neutralization titers as BA.1 and BA.5 in these cohorts (**Supplemental Fig. 2**).

65 In the BA.5-containing bivalent booster cohort, the neutralizing activity improved against all of
66 the Omicron subvariants (**Fig 1C**). The FRNT₅₀ GMTs were 2312 for WA1/2020, 618 for BA.1,
67 576 for BA.5, 201 for BA.2.75.2 and 112 for BQ.1.1. Relative to WA1/2020, this corresponded to
68 a reduction in neutralization titers of 4-fold against BA.1 and BA.5 and 11- and 21- fold against
69 BA.2.75.2 and BQ.1.1, respectively.

70 Individuals that received either one or two monovalent COVID-19 boosters had a dramatic
71 decrease in neutralization activity against Omicron subvariants compared to WA1/2020. This
72 decrease was especially profound for BA.2.75.2 and BQ.1.1, which contain the predicted
73 escape mutation R346T. Individuals that received the BA.5-containing bivalent booster showed
74 improved neutralizing activity against all Omicron subvariants. These responses are similar to
75 recent observations in individuals with breakthrough Omicron infection showing broadened
76 neutralizing activity against Omicron variants⁶. Limitations of this study include small cohort size,
77 unknown impact of prior SARS-CoV-2 exposure, and examination of a single timepoint. These
78 data demonstrate an overall serological benefit of bivalent booster immunizations.

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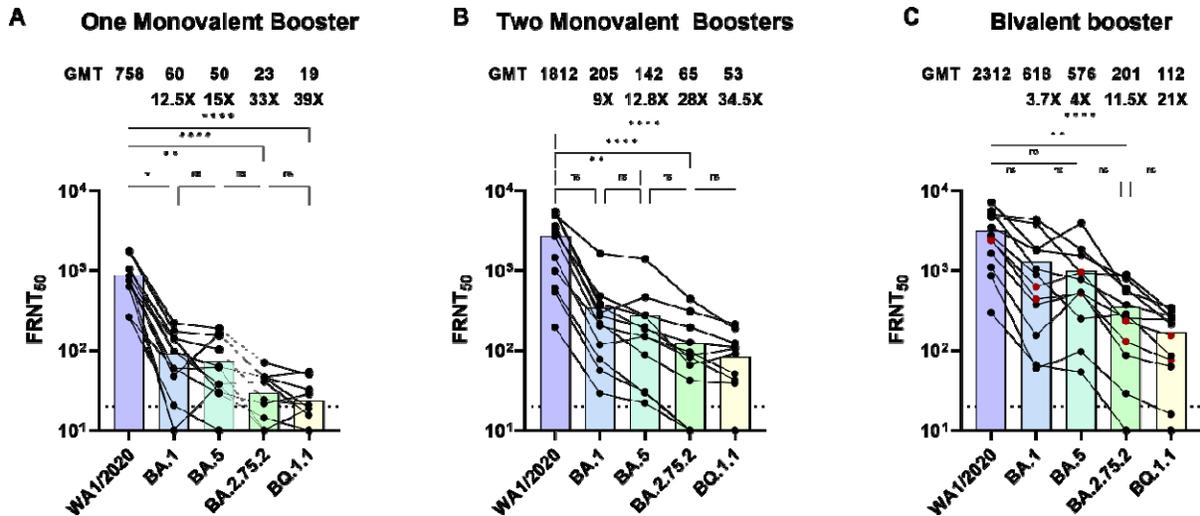
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93 **Figure Legend**

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97 **Figure 1. Neutralizing responses against WA1/2020, BA.1, BA.5, BA.2.75.2, and BQ.1.1.**

98 Shown is the neutralization activity against SARS-CoV-2 variants among 12 individuals who
99 received one monovalent booster (Panel A), 12 individuals of received two monovalent boosters
100 (Panel B), and 12 individuals who received the updated bivalent booster (Panel C). The focus
101 reduction neutralization test (FRNT₅₀ [the reciprocal dilution of serum that neutralizes 50% of the
102 input virus]) geometric mean titers for each variant are shown above each panel along with
103 ratios of GMT compared to WA1/2020. The connecting lines between the variants represent
104 matched serum samples. The horizontal lines represent the limit of detection of the assay
105 (FRNT₅₀ GMT 20). Red symbols in panel C indicate two individuals self-reported prior SARS-
106 CoV-2 infection. The differences between all groups were determined with the Kruskal–Wallis
107 test with Dunn’s correction for multiple comparisons. * p<0.05, ** p<0.01, ***p<0.001, ****
108 p<0.0001, n.s not significant.

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