

1 **Antibody responses to Omicron BA.4/BA.5 bivalent mRNA vaccine booster shot**

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15 **Abstract**

16

17 The SARS-CoV-2 Omicron variant and its numerous sub-lineages have exhibited a striking
18 ability to evade humoral immune responses induced by prior vaccination or infection. The Food
19 and Drug Administration (FDA) has recently granted Emergency Use Authorizations (EUAs) to
20 new bivalent formulations of the original Moderna and Pfizer mRNA SARS-CoV-2 vaccines that
21 target both the ancestral strain as well as the Omicron BA.4/BA.5 variant. Despite their
22 widespread use as a vaccine boost, little is known about the antibody responses induced in
23 humans. Here, we collected sera from several clinical cohorts: individuals after three or four
24 doses of the original monovalent mRNA vaccines, individuals receiving the new bivalent
25 vaccines as a fourth dose, and individuals with BA.4/BA.5 breakthrough infection following
26 mRNA vaccination. Using pseudovirus neutralization assays, these sera were tested for
27 neutralization against an ancestral SARS-CoV-2 strain, several Omicron sub-lineages, and
28 several related sarbecoviruses. At ~3-5 weeks post booster shot, individuals who received a
29 fourth vaccine dose with a bivalent mRNA vaccine targeting BA.4/BA.5 had similar neutralizing
30 antibody titers as those receiving a fourth monovalent mRNA vaccine against all SARS-CoV-2
31 variants tested, including BA.4/BA.5. Those who received a fourth monovalent vaccine dose had
32 a slightly higher neutralizing antibody titers than those who received the bivalent vaccine against
33 three related sarbecoviruses: SARS-CoV, GD-Pangolin, and WIV1. When given as a fourth
34 dose, a bivalent mRNA vaccine targeting Omicron BA.4/BA.5 and an ancestral SARS-CoV-2
35 strain did not induce superior neutralizing antibody responses in humans, at the time period
36 tested, compared to the original monovalent vaccine formulation.

37 **Main text**

38
39 Continued evolution of Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2), which causes
40 Coronavirus Disease 2019 (COVID-19), has led to the emergence of the Omicron variant and
41 numerous sub-lineages that evade neutralizing antibody responses induced by infection or
42 vaccination¹. In response to this concerning trend, the Food and Drug Administration granted
43 Emergency Use Authorizations (EUAs) to bivalent formulations of mRNA vaccines produced by
44 Pfizer and Moderna that target both the Omicron BA.4/BA.5 spike and an ancestral wild-type
45 (WT) SARS-CoV-2 spike². Published data on antibody responses to bivalent vaccines have been
46 limited to animal studies and human studies utilizing a different bivalent mRNA vaccine
47 targeting the Omicron BA.1 spike in addition to the WT spike^{3,4}. Despite their widespread use,
48 the impact of a booster shot with a new bivalent vaccine on SARS-CoV-2-neutralizing antibody
49 responses in humans remains unknown.

50
51 Therefore, we collected a panel of sera from individuals who had received three doses of the
52 original monovalent mRNA vaccines followed by one dose of a bivalent vaccine targeting
53 BA.4/BA.5 (details in Supplementary Appendix). We compared virus neutralization by these
54 sera to panels of sera from individuals who received either three or four monovalent mRNA
55 vaccines as well as to sera from individuals with BA.4/BA.5 breakthrough infection following
56 mRNA vaccination. Using pseudovirus neutralization assays, all sera were tested against an
57 ancestral SARS-CoV-2 strain (D614G) and Omicron sub-lineages BA.1, BA.2, BA.4/BA.5,
58 BA.4.6, BA.2.75, and BA.2.75.2. To further assess the breadth of antibody responses, we also
59 tested sera for neutralization against several related sarbecoviruses: SARS-CoV, GD-pangolin,
60 GX-pangolin, and WIV1.

61
62 Clinical details are summarized for all groups in **Table S1** and listed for each case in **Table S2**.
63 Individuals who received four monovalent mRNA doses were older (mean age 55.3) than those
64 who received a bivalent booster (mean age 36.4). Serum was collected from both cohorts at a
65 similar time following the vaccine boost (mean 24.0 days in the monovalent group; mean 26.4
66 days in the bivalent group). All cohorts exhibited the highest serum virus-neutralization titers
67 (ID₅₀) against the ancestral D614G strain (**Figure 1A**). Geometric mean ID₅₀ titers against
68 SARS-CoV-2 variants were lowest for boosted sera and highest for BA.4/BA.5 breakthrough
69 sera. There was no significant difference in neutralization of any SARS-CoV-2 variant tested
70 between individuals who received a fourth monovalent vaccine and those who received a fourth
71 dose of a bivalent vaccine (**Figure 1B**). ID₅₀ titers against three related sarbecoviruses (SARS-
72 CoV, GD-Pangolin, and WIV1) were slightly but significantly higher in those who received a
73 fourth monovalent vaccine dose compared to those who received a bivalent vaccine.

74
75 Boosting with a new bivalent mRNA vaccine targeting both BA.4/BA.5 and an ancestral SARS-
76 CoV-2 strain did not elicit a discernibly superior virus-neutralizing antibody responses compared

77 boosting with an original monovalent vaccine. These findings may be indicative of
78 immunological imprinting⁵, although follow-up studies are needed to determine if the antibody
79 responses will deviate in time, including the impact of a second bivalent booster.

80 **Figure 1. Neutralization profiles of serum samples against SARS-CoV-2 variants and other**
81 **sarbecoviruses.**

82 (A) Neutralization ID₅₀ titers of serum samples from “3 shots WT”, “BA.4/BA.5 breakthrough”,
83 “4 shots WT”, and “3 shots WT + bivalent” cohorts. Values above the symbols denote the
84 geometric mean ID₅₀ titers, and values on the lower left indicate the sample size (n). The limit of
85 detection is 100 (dotted line). Wild-type (WT) shots refer to monovalent mRNA vaccine doses.
86 “3 shots WT,” sera from individuals vaccinated with three doses of the WT mRNA vaccine;
87 “BA.4/BA.5 breakthrough,” sera from individuals with BA.4 or BA.5 infection following WT
88 mRNA vaccination; “4 shots WT,” sera from individuals vaccinated with four doses of the WT
89 mRNA vaccine; “3 shots WT + bivalent,” sera from individuals vaccinated with three doses of
90 the WT mRNA vaccine and subsequently one dose of a BA.4/BA.5 bivalent mRNA vaccine. (B)
91 Comparison of antibody responses induced by a fourth dose of the original WT mRNA vaccine
92 versus a fourth dose of a BA.4/BA.5 bivalent mRNA vaccine. Comparisons were made by
93 Mann-Whitney tests. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Values above the symbols denote the
94 geometric mean ID₅₀ titers.

95 **References**

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