High neutralizing antibody levels against SARS-CoV-2 Omicron after UB-612 booster vaccination

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Running title: UB-612 boosted NAbs against Omicron

Key words: SARS-CoV-2, COVID-19, vaccine, spike protein, RBD, antibody, neutralizing antibody, clinical trial

Abstract

Omicron, a highly transmissible SARS-CoV-2, emerged in November 2021. The high mutation rates within spike protein of Omicron raised concerns about increased breakthrough infections among the vaccinated. We tested cross-reactivity of antibodies induced by UB-612 against Omicron and other variants. After 2 doses, UB-612 elicited low levels of neutralization antibodies against ancestral virus and Omicron. A booster dose delivered 7-9 months after primary vaccination dramatically increased antibody levels, with only a 1.4-fold loss in neutralization titer against Omicron compared to the ancestral strain. Using a model bridging vaccine efficacy with ancestral virus RBD binding antibody responses, predicted efficacy against symptomatic COVID-19 after UB-612 booster is estimated at 95%. UB-612 is anticipated to be a potent booster against current and emerging SARS-CoV-2 variants.

One-Sentence Summary: UB-612 booster induced broadly neutralizing antibodies against Omicron and is presumed to be protective against COVID-19.

Main Text:

In November 2021, the Omicron (B.1.1.529) Variant of Concern (VOC) was first detected in South Africa and quickly spread globally, becoming the dominant SARS-CoV-2 variant worldwide. Omicron's high transmissibility and potential for immune system evasion, as suggested by its ability to infect and be transmitted by previously infected and vaccinated individuals, predicts a transmission advantage over the Delta variant and the displacement of the latter as the dominant variant (*1*).

The Omicron variant (BA.1 and BA.2 lineages) has over 50 new mutations, >15 of which are in the receptor-binding domain (RBD) of the Spike (S) protein (*2*, *3*). Given that over 90% of neutralizing antibodies present in plasma of convalescent individuals and up to 99% of neutralizing antibodies elicited by vaccination with the mRNA-1273 vaccine are directed to the RBD (*4*), these mutations could be largely responsible for Omicron's ability to evade neutralizing antibodies induced by the approved COVID-19 vaccines (*5-8*). Multiple studies have shown a 20- to 30-fold reduction in neutralization antibody activity against Omicron in the sera of primary vaccine recipients compared with the ancestral SARS-CoV-2 or D614G viruses (5-*7,9-12*). The emergence of new variants, including Omicron, in addition to the rapidly waning immunity of vaccines over time has raised concerns about breakthrough infections in vaccinated individuals, and highlighted the need for booster doses worldwide. Homologous or heterologous booster vaccines, all based on the full-length S protein, restored protective neutralizing antibodies to levels achieved by the primary immunization; however, these titers were 7.1-fold lower against Omicron than the ancestral strain, suggesting a continued risk of breakthrough infections in vaccinated individuals over time (*6*).

In contrast to most of the approved COVID-19 vaccines that encode the full-length S protein, the UB-612 vaccine candidate is composed of Wuhan-Hu S1-RBD-sFc fusion protein and is enriched with 5 rationally designed peptides representing Sarbecovirus conserved Th and CTL epitopes on the S2 subunit, Membrane (M), and Nucleocapsid (N) proteins (*13*). A favorable safety and tolerability profile for UB-612 was demonstrated in ~4000 participants in a Phase 1 trial and its extension and a Phase 2 trial conducted in Taiwan *(14)*. In both Phase 1 and Phase 2 trials, the UB-612 vaccine was found to have a favorable safety profile and low reactogenicity after every injected dose. Two immunizations with UB-612 were immunogenic and led to seroconversion rate of neutralizing antibody in >90% of vaccine recipients. In these same studies, UB-612 was shown to elicit long-lasting neutralizing antibody titers similar to levels detected in convalescent patients (*15*) and B cell and T cell responses against Delta and Omicron variants *(14)*.

The objectives of this study were to evaluate the neutralization potential of antibodies elicited by a third dose (booster) with the RBD-based vaccine UB-612 against Omicron and their reactivity to recombinant S and RBD protein antigens across various variants.

After receiving a 2-dose primary vaccine series or a booster given at 7-9 months after the second dose, sera from 15 participants from Phase 1 (UB-612, 100-µg dose) were tested in a live virus neutralization test (VNT) at Vismederi, Siena, Italy (a Coalition for Epidemic Preparedness Innovations central testing laboratory for COVID-19 vaccines) **(Table S1)**. Previously, to establish an International Reference Standard for anti−SARS-CoV-2 antibody detection, the VNT used in our analysis and performed by Vismederi, was compared with other VNTs and found to be the most stringent assay, resulting in a lower geometric mean titer (GMT) than other plaque reduction-, foci reduction-, cytopathic effect (CPE)-, or pseudotyped virus-based neutralization assays (*16*).

Two doses of UB-612 showed modest neutralizing activity against the ancestral strain (2019-nCov/Italy-INMI1) (GMT VNT_{50} of 13.2), and activity slightly above the level of detection against Omicron (GMT VNT_{50} of 11) (**Fig. 1**). In comparison, two dose immunization with mRNA vaccines resulted in similarly low levels of Omicron neutralizing antibody responses: (i) mRNA-1273 on day 21 after immunization stimulated GMT $pVNT_{50}$ of 14, and (ii) BNT162b2 on day 28 after immunization lead to GMTs VNT of 7 (*7*, *11*). A third booster dose of UB-612 delivered 7-9 months after the primary series increased neutralizing antibody titers against Omicron and the ancestral strain to 670 and 970, respectively, which constitutes a 61- and 73-fold higher GMT than those achieved after 2 doses **(Fig. 1)**. Notably, the estimated loss in neutralization titer against Omicron in sera obtained 2 weeks after the booster was only 1.4-fold compared to the ancestral strain in a live virus assay and 5.5-fold in a pseudovirus assay *(14*). In contrast, after the third booster dose, the neutralizing antibody GMT against Omicron and ancestral strain were 106 and 763 (6.3-fold loss) for BNT162b2 (*11*), or 850 and 2423 (2.9-fold loss) for mRNA-1273-50 μg (*7*). This additional evidence supports the greater extent of UB-612−elicited neutralizing antibodies across multiple SARS-CoV-2 variants particularly after 3 doses, a differentiation property of UB-612 primarily attributed to its RBD antigenic component (*15*).

To evaluate reactivity of UB-612−elicited antibodies to S and RBD protein antigens, we tested sera from the Phase 1 trial participants and from 84 randomly selected Phase 2 trial participants immunized with UB-612 (**Tables S1-S2**) with 2 ELISA-based assays for immunoglobulin G (IgG) direct binding to recombinant S and RBD protein antigens and inhibition of recombinant S and RBD protein binding to the human angiotensin-converting enzyme 2 (hACE2) receptor. A third dose of UB-612 booster immunization stimulated broadly

reactive IgG antibodies, effectively binding to RBDs of 14 divergent SARS-CoV-2 variants, including Alpha, Beta, Gamma, Delta, and Omicron (**Fig. 2** and **Fig. S1**).

Compared with the second UB-612 dose, IgG binding titers against Omicron's RBD increased by over 40-fold, and the titers against RBDs of other SARS-CoV-2 variants were also increased in the range of 30- to 50-fold after the booster dose. When the IgG titer ratio (in BAU/mL) of several variants was compared to the ancestral Wuhan strain, the normalized RBD antibody-binding responses to the tested variants were found to be similar after 2 or 3 doses: Alpha (0.98-fold), Beta (2.44-fold), Delta (1.33-fold), Gamma (1.77-fold), and Omicron (3.3-fold), after 2 doses; and Alpha (0.91-fold), Beta (1.8-fold), Delta (1.4-fold), Gamma (1.55-fold), and Omicron (3.7-fold), after 3 doses.

Similar to RBD binding, the results of the S-protein binding antibody responses (S:ACE2- and RBD:ACE2-blocking antibody titers), confirmed the extent of stability in ratios of parental to variant IgG antibodies stimulated by 2 or 3 doses of UB-612, despite an up to 60-fold increase in titers against different variants after the booster dose (**Fig. S2, Fig. S3**).

We also compared the level of UB-612−elicited IgG antibodies with data previously reported for several authorized vaccines determined in equivalent S- and RBD-binding assays (*17*). After a 2-dose primary immunization series, the GMTs of UB-612−elicited IgG antibodies were 69 and 127 (BAU/mL) against the Wuhan S protein, and 235 and 494 (BAU/mL) against the RBD antigen in sera from Phase 1 and Phase 2 participants, respectively (**Fig. S4**). These IgG responses were comparable to those observed in individuals after the primary immunization with adenovirus vectored vaccines (1-dose Ad26.COV2.S or 2-dose ChAdOx1-S), but were lower than the response observed after 2 immunizations with mRNA vaccines. The additional booster dose with UB-612 increased levels of both S- and RBD-protein binding IgG antibodies in the

Phase 1 participants by more than 16- and 13-fold, and increased antibody GMTs to 2138 and 6767 (BAU/mL), respectively, matching those achieved by 2 immunizations with the mRNA vaccines.

The homologous booster dose of mRNA-1273, BNT162b2, or Ad26.COV.S S-based vaccines dramatically increased neutralizing antibodies to Omicron (20- to 30-fold) compared to the modest increase reported for the ancestral strain (1- to 4-fold) (*18*). The vaccination with UB-612 elicited highly cross-reactive IgG and neutralizing antibodies to variants including Omicron and the ratio of ancestral strain/Omicron and other variants remained stable after the second and booster immunizations. It was demonstrated that a booster with a full-length S protein vaccine would refocus/recall the memory B cell pool to produce neutralizing antibodies to conserved RBD regions that have been affinity-matured after a long interval between the doses, enhancing the breadth of cross-variant neutralization (*19*). We believe that the UB-612 vaccine may be able to recall such memory B cell responses targeting the RBD region carrying the major neutralizing epitopes.

We further established a vaccine efficacy prediction model based on the RBD activity of IgG antibodies to the ancestral strain, extending previous models based on neutralizing antibodies (*20, 21*) or S protein−binding activities *(17)*. According to this model, the predicted vaccine efficacy of UB-612 against symptomatic disease caused by the prototype strain is ~80% for 2 doses (235 BAU/mL sera from for 15 Phase 1 participants and 494 BAU/mL from 84 randomly selected Phase 2 participants, respectively, **Fig. S4**) and ~95% after the booster dose (6,767 BAU/mL) (**Fig. 3**).

In summary, a third booster dose of UB-612 elicited robust IgG binding and neutralizing antibodies against several SARS-CoV-2 variants, including Omicron. The magnitude and extent

of reactivity of the humoral response after the UB-612 booster match those reported for the authorized vaccines, including mRNA-1273. Additionally, UB-612 immunization has been shown to stimulate T-cell responses against conserved S, N, and M Th/CTL peptides, included in the UB-612 vaccine formulation *(13,14, 22)*, and may provide long-lasting antibody responses (*15*) which further differentiates UB-612 from many authorized vaccines. As SARS-CoV-2 continues to evolve, several strategies are being explored to effectively prevent COVID-19 caused by newly emerging SARS-CoV-2 variants, including monovalent variant antigen matching, multivalent, or universal vaccine approaches. Our results indicate that UB-612 could offer an alternative strategy for the rapid development of a booster vaccine, offering high levels of antibody responses with extensive activity across currently circulating and potentially future SARS-CoV-2 variants.

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Acknowledgments

We thank many colleagues at UBIAsia, UBP, and Vaxxinity who designed, developed, and produced the UB-612 vaccine candidate and performed the Phase 1 and 2 clinical trials in Taiwan. We are also grateful to the Vismederi team for their work on live virus-neutralizing antibody assays.

Funding

This wok was supported by Vaxxinity Inc

Author contributions

FG: conceived and conceptualized, wrote the manuscript

SW: analyzed data, interpreted data, wrote the manuscript

CYW, WJP, HL, HKK: consented the patients for the samples to be used for this study and shipped serum samples for binding and neutralization assays and reviewed the manuscript

MMH, TPM: assisted in planning of the study

AR: wrote and reviewed the manuscript

LW: analyzed data

DG, MG, AH: performed experiments and interpreted data

Competing interests

FG, SW, LW, MMH, TM and AR are employees at Vaxxinity, Inc, Dallas, TX, USA.

CYW, WJP, HKK and HL are employees at United Biomedical Inc Asia, Taipei, Taiwan.

Data and material availability

The data supporting the findings of the study are available in the article and supplemental material. The raw data supporting the findings of this study and materials can be available from the corresponding author upon reasonable request.

Supplementary Materials

Materials and Methods

Figs. S1 to S4

Tables S1, S2

FIGURES

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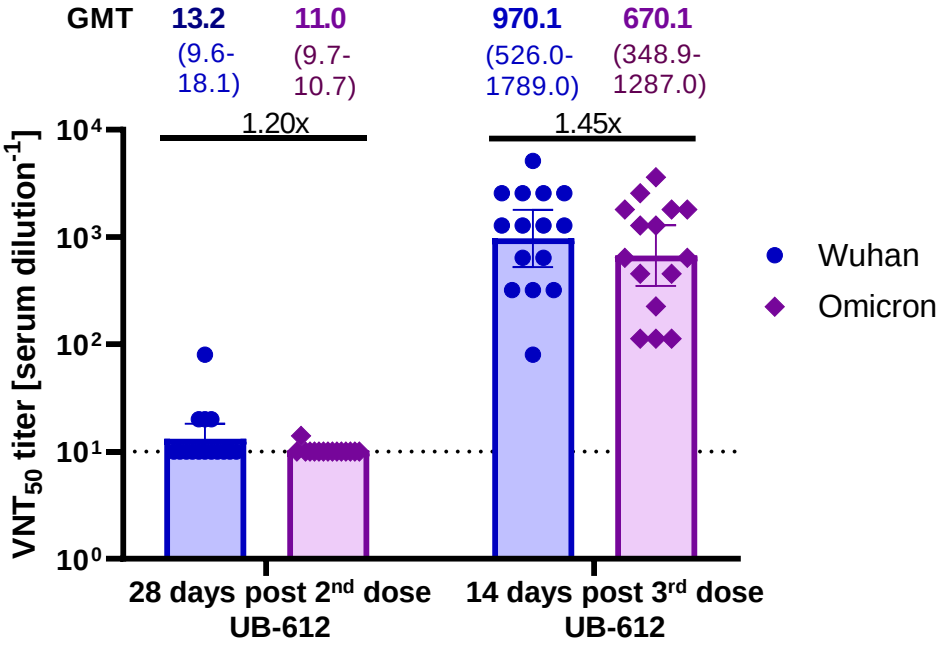

Fig. 1. Neutralizing antibodies against SARS-CoV-2 Wuhan strain (2019-nCoV/Italy-INMI1) and Omicron (B.1.1.529) variant in sera from Phase 1 trial participants (n=15) collected 28 days after 2 doses and 14 days after 3 doses with UB-612 (100 µg). Data expressed in the reciprocal dilutions for each serum sample and GMT (95% CI) are plotted. Of note, the lower level of antibodies against the ancestral strain compared with those obtained from a similar validated CPE assay performed using the same sera (GMT VNT_{50} of 127) (*15*) is likely to be assay specific.

GMT, geometric mean titers; LOD, limit of detection; VNT, virus neutralization test.

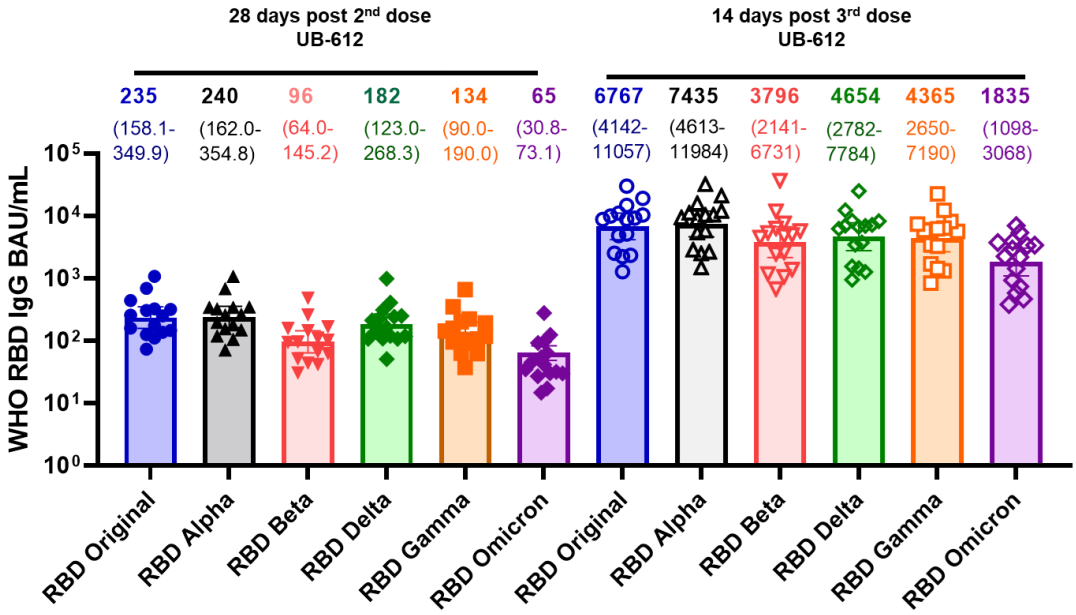

Fig. 2. IgG binding titers against SARS-CoV-2 major VOCs in sera collected 28 days after 2 doses and 14 days after 3 doses with UB-612 (100 µg) from Phase 1 trial participants (n=15). The loss of antibody binding to the RBD of variants compared with the original RBD (ancestral strain) remains stable between 2 doses and 3 doses of UB-612 vaccine, despite a high increase in levels of binding antibodies to RBD. The ratios of original RBD to variants are 0.9, 2.4, 1.3, 1.7 and 3.6 (after 2 doses) and 0.9, 1.8, 1.4, 1.5 and 3.7 (after booster, 3 doses), for Alpha, Beta, Delta, Gamma, and Omicron, respectively.

RBD, receptor-binding domain; VOC, Variant of Concern.

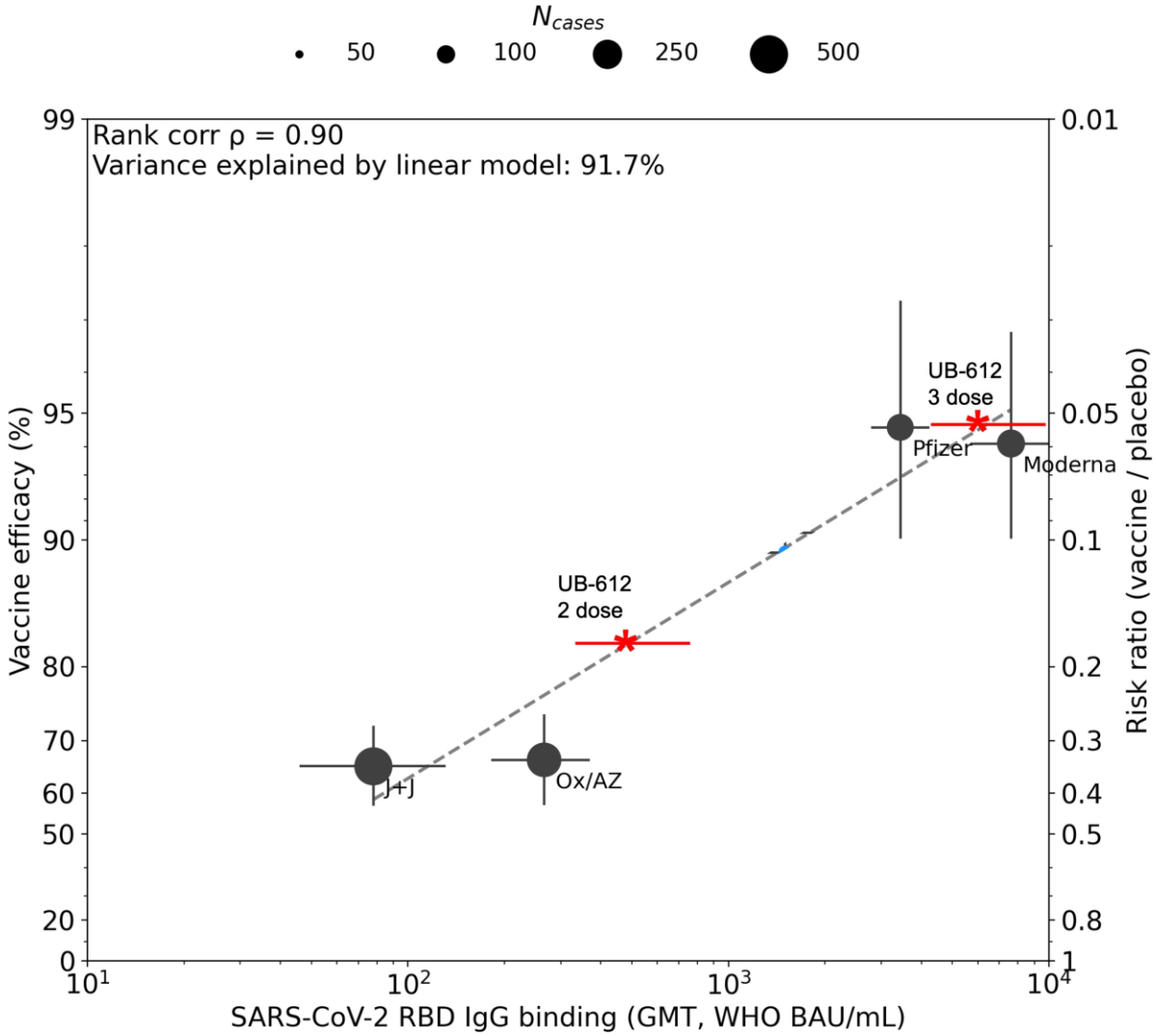

Fig. 3. Estimated UB-612 efficacy after 2 and 3 doses. A model bridging vaccine-induced RBD IgG response to vaccine efficacy against symptomatic COVID-19 caused by ancestral Wuhan *(17)*. Estimated efficacy of UB-612 after 2 doses is ~80% (CI, 80%-85%) based on RBD binding IgG antibodies (GMT 494 BAU/mL, 95% CI, 337-725), and ~95% (93%-97%) after a booster vaccination (GMT 6767 (95% CI, 4142-11,057).

IgG, immunoglobulin G; RBD, receptor-binding domain