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UB-612, A Multitope Universal Vaccine Eliciting a Balanced B and T cell Immunity against SARS-CoV-2 Variants of Concern

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Abstract

Importance: The SARS-CoV-2 non-spike structural proteins of Nucleocapsid (N), Membrane (M) and Envelope (E) are critical in the host cell interferon response and memory T-cell immunity and have been utterly overlooked in the development of COVID vaccines.

Objective: To determine the safety and immunogenicity of UB-612, a multitope vaccine containing S1-RBD-sFc protein and rationally-designed promiscuous peptides representing sequence-conserved Th and CTL epitopes on the Sarbecovirus Nucleocapsid (N), Membrane (M) and Spike (S2) proteins.

Design, setting and participants: UB-612 booster vaccination was conducted in Taiwan. A UB-612 booster dose was administered 6-8 months post-2nd dose in 1,478 vaccines from 3,844 healthy participants (aged 18 years to 85 years) who completed a prior placebo (saline)-controlled, randomized, observer-blind, multi-center Phase-2 primary 2-dose series (28 days apart) of UB-612. The interim safety and immunogenicity were evaluated until 14 days post-booster.

Exposure: Vaccination with a booster 3rd dose of UB-612 vaccine.

Main outcomes and measures: Solicited local and systemic AEs were recorded for seven days in the e-diaries of study participants, while skin allergic reactions were recorded for fourteen days. The primary immunogenicity endpoints included viral-neutralizing antibodies against live SARS-CoV-2 Wild-Type (WT, Wuhan strain) and live Delta variant (VNT50), and against pseudovirus WT and Omicron variant ($pVNT_{50}$). The secondary immunogenicity endpoints included anti-S1-RBD IgG antibody, S1-RBD: ACE2 binding inhibition, and T-cell responses by ELISpot and Intracellular Cytokine Staining (ICS).

Results: No post-booster vaccine-related serious adverse events were recorded. The most common solicited adverse events were injection site pain and fatigue, mostly mild and transient. The UB-612 booster prompted a striking upsurge of neutralizing antibodies against live WT Wuhan strain (VNT_{50}, 1,711) associated with unusually high cross-neutralization against Delta variant (VNT_{50}, 1,282); and similarly with a strong effect against pseudo virus WT ($pVNT_{50}$, 6,245) and Omicron variant ($pVNT_{50}$, 1,196). Upon boosting, the lower VNT_{50} and $pVNT_{50}$ titers of the elderly in the primary series were uplifted to the same levels as those of the young adults. The UB-612 also induced robust, durable VoC antigen-specific Th1-oriented (IFN-γ^+-) responses along with $CD8^+$ T-cell ($CD107a^+$-Granzyme B^+) cytotoxicity.

Conclusions and relevance: With a pronounced cross-reactive booster effect on B and T-cell immunity, UB-612 may serve as a universal vaccine primer and/or booster for comprehensive immunity enhancement against emergent VoCs.

Trial registration: ClinicalTrials.gov: NCT04773067

Keywords: UB-612 • Multitope Universal Vaccine • Booster vaccination • SARS-CoV-2 • Sarbecovirus • Variants of Concern

Key points:

Question: Facing ever-emergent SARS-CoV-2 variants and long-haul COVID, can composition-innovated new vaccines be constructed capable of inducing striking, broad and durable booster-recalled B/T-immunity to prevent infection by VoCs?

Findings: In a Phase-2 extension study, a booster dose of UB-612 multitope protein-peptide vaccine prompted high viral-neutralizing titers against live wild-type virus (VNT_{50}, 1,711), Delta variant (VNT_{50}, 1,282); pseudovirus wild-type ($pVNT_{50}$, 6,245) and Omicron variant ($pVNT_{50}$, 1,196). Robust, durable Th1-IFNγ^+ responses and $CD8^+$ T cell-($CD107a^+$-Granzyme B^+) cytotoxic activity were both observed.

Meaning: UB-612 RBD-sFc vaccine armed with T cell immunity-promoting conserved N, M and S2 Th/CTL epitope peptides may serve as a universal vaccine to fend off new VoCs.

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Introduction

The devastation of the virulent delta and the hyper-transmissible, pathologically-lesser Omicron variants (Supplementary Figures 1A and 1B) has declined and COVID restrictions have been lifted [1-11]. While mRNA booster vaccination could reverse Omicron-induced decrease of serum neutralizing antibodies and reduce rates of hospitalization and severe disease, it offers less protection against infection and mild disease. Still, Omicron (BA.1; BA.1.1.529). can cause breakthrough infections even after the fourth vaccine jab [11].

Omicron-infected asymptomatic and mild cases shall not be treated akin to flu. Reportedly, COVID can take a serious toll on heart health presumably to stay as a part of long-haul COVID [12,13]. This toll, beyond the myocarditis and pericarditis associated with mRNA vaccines [14], encompasses a wide range of inflammatory cardiovascular disorders that elevates, depending on COVID severity, from asymptomatic, symptomatic, to acute infection cases [12,15,16].

Facing ever-emergent variants and long-haul COVID, new vaccines that can prevent infection are strongly advocated [17,18]. A universal vaccine capable of inducing durable cross-reactive viral-neutralizing antibodies along with broad T-cell immunity is desirable. Apparently, the currently authorized spike-only vaccines do not incorporate SARS-CoV-2's non-spike structure proteins of Envelope (E), Membrane (M) and Nucleocapsid (N), the regions critically involved in the host cell interferon response and T-cell memory. The non-spike structure proteins are utterly overlooked in COVID vaccine development.

Here we report the booster immunogenicity and safety in Phase-2 trial of UB-612, a multitope vaccine [23]. containing an S1-RBD-sFc protein and rationally-designed Th and CTL epitope peptides on the Sarbecovirus Nucleocapsid (N), Membrane (M) and Spike (S2) proteins that are sequence-conserved across all VoCs (Figure 1) (Table 1) [23].

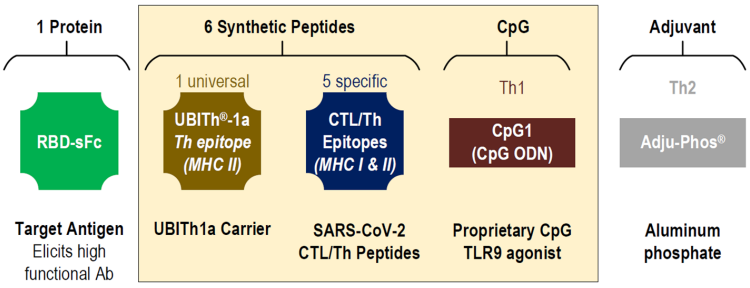

Figure 1. Components of UB-612 multitope protein-peptide vaccine with highly conserved T cell epitope peptides to achieve balanced humoral and cellular immunity. UB-612 vaccine construct contains an S1-RBD-sFc single chain fusion protein targeting only the receptor ACE2 conformational binding site for the B cell epitopes, plus five synthetic Th/CTL peptides derived from SARS-CoV-2 Nucleocapsid (N), Membrane (M) and spike S2 proteins, and the proprietary UBITh1a peptide as a T cell activation catalyst, all with promiscuous MHC (HLA) class I and II binding motifs with broad MHC (HLA) genetic coverage, and known to bind and trigger T cell proliferation. These positively charged designer T peptides are bound to our proprietary highly negatively charged CpG1 through charge neutralization, which is then bound to Adjuphos® as an adjuvant to constitute the UB-612 vaccine drug product.

Table 1. Highly conserved Th/CTL epitopes on membrane (M), nucleocapsid (N), and spike-2 (S2) proteins across SARS-CoV-2 Variants of Concern (VoC[a]).

Wild type and VoCs	M protein SARS-CoV2 M101-156 CTL epitope	N protein SARS-CoV2 N305-331 Th/CTL epitopes	S2 protein SARS-CoV2 S957-984 (Th/CTL epitopes)	S2 Protein SARS-CoV2 S891-917 (Th epitope)	S2 Protein SARS-CoV2 S996-1028 (Th/CTL epitope)
Wuhan (Original)	GLMWLSYFIASFRLFARTR SMWS	AQFAPSASAFFGMS RIGMEVTPSGTWL	QALNTLVKQLSSNF GAISSVLNDILSRL	GAALQIPFAMQMAYRFN GIGVTQNVLY	LITGRLQSLQTVVTQLIR AAEIRASANLAATK
Alpha (B1.1.7) UK	GLMWLSYFIASFRLFARTR SMWS	AQFAPSASAFFGMS RIGMEVTPSGTWL	QALNTLVKQLSSNF GAISSVLNDILSRL	GAALQIPFAMQMAYRFN GIGVTQNVLY	LITGRLQSLQTVVTQLIR AAEIRASANLAATK
Beta (B.1.351) South Africa	GLMWLSYFIASFRLFARTR SMWS	AQFAPSASAFFGMS RIGMEVTPSGTWL	QALNTLVKQLSSNF GAISSVLNDILSRL	GAALQIPFAMQMAYRFN GIGVTQNVLY	LITGRLQSLQTVVTQLIR AAEIRASANLAATK
Gamma (P.1) Brazil	GLMWLSYFIASFRLFARTR SMWS	AQFAPSASAFFGMS RIGMEVTPSGTWL	QALNTLVKQLSSNF GAISSVLNDILSRL	GAALQIPFAMQMAYRFN GIGVTQNVLY	LITGRLQSLQTVVTQLIR AAEIRSANLAATTKA
Delta[b] (B.1.617.2) India	GLMWLSYFIASFRLFARTR SMWS	AQFAPSASAFFGMS RIGMEVTPSGTWL	QALNTLVKQLSSNF GAISSVLNDILSRL	GAALQIPFAMQMAYRFN GIGVTQNVLY	LITGRLQSLQTVVTQLIR AAEIRASANLAATK
Omicron[c] (B.1.1.529; BA.1) Botswana South Africa	GLMWLSYFIASFRLFARTR SMWS	AQFAPSASAFFGMS RIGMEVTPSGTWL	QALNTLVKQLSSKF GAISSVLNDIFSRL	GAALQIPFAMQMAYRFN GIGVTQNVLY	LITGRLQSLQTVVTQLIR AAEIRASANLAATK

Note: [a] The presence of T cell epitopes is critical for the induction of B and T cell memory responses against viral antigens. SARS-CoV-2 CTL and Th epitopes, validated by HLA binding and T cell functional assays, are highly conserved between SARS-CoV-2 (2019) and SARS-CoV-1 (2003) by sequence alignment, with minute between-variant differences seen only in S957-984 and S891-917.

[b] None of the five designer epitope peptides in the M, N, and S2x3 proteins has an aa-residue that overlaps with the mutation sites reported for the Delta variant (https://en.wikipedia.org/wiki/SARS-CoV-2_Delta_variant).

[c] Except for N969K, L981F within S957-984 peptide on the S2 spike protein, none of the other four designer epitope peptides in the M, N, and S2 proteins has an aa-residue that overlaps with the mutation sites reported for the Omicron variant (https://en.wikipedia.org/wiki/SARS-CoV-2_Omicron_variant).

Materials and Methods

Design of extension booster trial, objectives, and oversight

Booster 3rd-dose following the phase-2 trial primary 2-dose series: We conducted a booster vaccination study (n=1,478) which was an extension arm of the phase-2, placebo-controlled, randomized, observer-blind, multi-center primary 2-dose study (ClinicalTrials.gov: NCT04773067) in Taiwan with 3,844 healthy male or female adults aged>18 years to 85 years who received two intramuscular doses (28 days apart) of 100 μg UB-612 or saline placebo [24]. The objectives of the third-dose extension study were to determine the booster-induced safety and immunogenicity after unbinding, 6 to 8 months after the second dose.

The Principal Investigators at the study sites agreed to conduct the study according to the specifics of the study protocol and the principles of Good Clinical Practice (GCP); and all the investigators assured accuracy and completeness of the data and analyses presented. The protocols were approved by the ethics committee at the site and all participants provided written informed consent. Full details of the booster trial design, inclusion and exclusion criteria, conduct, oversight, and statistical analysis plan are available in Supplementary (Supplementary Figure 2) (Supplementary Table 1).

Vaccine product and placebo

Like the same vaccine product used in the Phase-1 primary and booster and the Phase-2 primary trials, UB-612 vaccine used in the present phase-2 extension booster vaccination is a multitope vaccine designed to activate both humoral and cellular responses. For SARS-CoV-2 immunogens, UB-612 combines a CHO-expressed S1-RBD-sFc fusion protein (Wuhan strain) and a mixture of synthetic T helper (Th) and Cytotoxic T Lymphocyte (CTL) epitope peptides, which are selected from immunodominant M, S2 and N regions known to bind to human Major Histocompatibility Complexes (MHC) I and II. The preparation of UB-612 vaccine product consists of compounding, filtration, mixing, and filling operations. Before addition of the subunit protein S1-RBD-sFc, the individual components of the vaccine are filtered through a 0.22 micron membrane filter, including the peptide solution (2 μg/mL), CpG1, a proprietary Oligonucleotide (ODN), solution (2 μg/mL), 10X protein buffer containing 40 mM Histidine, 500 mM Arginine and 0.6% Tween 80, 20% sodium chloride stock solution. After sequentially addition of each component, the S1-RBD-sFc fusion protein and peptides are formulated with components described as above to form a protein-peptide complex and then is adsorbed to aluminum phosphate (Adju-Phos®) adjuvant. The last step would be addition of water for injection containing the 2-phenoxyethanol preservative solution to make final drug product at 200 μg/mL. The UB-612 vaccine product is stored at 2 to 8°C. Placebo used in the Phase-2 trial was sterile 0.9% normal saline.

Trial procedures and safety

Phase-2 trial of primary and booster third-dose series: The primary safety endpoints the Phase-2 trial was to evaluate the safety and tolerability of all participants receiving study intervention from Days 1 to 365. Vital signs were assessed before and after each injection. Participants were observed for 30 minutes after each injection for changes in vital signs or any acute anaphylactic reactions. After each injection, participants had to record solicited local and systemic AEs in their self-evaluation e-diary for up to seven days while skin allergic reactions were recorded in their e-diary for up to fourteen days. Safety endpoints include unsolicited AEs reported for Days 1 to Day 57 in this interim Phase-2 report. Complete details for solicited reactions are provided in the study protocols.

Scope of immunogenicity investigation

The primary immunogenicity endpoints were the Geometric Mean Titers (GMT) of neutralizing antibodies against SARS-CoV-2 Wild-Type (WT, Wuhan strain), and the post-booster effects against Omicron and Delta variant were explored as well. For WT and Delta strains, viral-neutralizing antibody titers that neutralize 50% (VNT_{50}) of live SARS-CoV-2 WT and Delta variant were measured by a Cytopathic Effect (CPE) based assay using Vero-E6 (ATCC® CRL-1586) cells challenged with SARS-CoV-2-Taiwan-CDC#4 (Wuhan strain) and SARS-CoV-2-Taiwan-CDC#1144 (B.1.617.2; Delta variant). The replicating virus neutralization test conducted at Academia Sinica was fully validated using internal reference controls and results expressed as VNT_{50}. The WHO reference standard was also employed and results reported in international units (IU/mL). For WT and Omicron strains, 50% pseudovirus neutralization titers ($pVNT_{50}$) were measured by neutralization assay using HEK-293T-ACE2 cells challenged with SARS-CoV-2 pseudovirus variants expressed the spike protein of WT and Omicron BA.1variants. Assay methods are detailed below.

The secondary immunogenicity endpoints include anti-S1-RBD IgG antibody, inhibitory titers against S1-RBD: ACE2 interaction, and T-cell responses assayed by ELISpot and Intracellular Staining. The RBD IgG ELISA was fully validated using internal reference controls and results expressed in end-point titers. The WHO reference standard was also employed and results reported in Binding Antibody Units (BAU/mL) (70, Lancet 2021; 397(10282):1347-1348.). A panel of 20 human convalescent serum samples from COVID-19 Taiwan hospitalized patients aged 20 years to 55 years were also tested for comparison with those in the vaccines. Human Peripheral Blood Mononuclear Cells (PBMCs) were used for monitoring T cell responses. All bioassay methods are detailed below:

Viral-neutralizing antibody titers against SARS-CoV-2 wild-type and variants by CPE based live virus neutralization assay: Neutralizing antibody titers were measured by CPE-based live virus neutralization assay using Vero-E6 cells challenged with wild type (SARS-CoV-2-Taiwan-CDC#4, Wuhan) and Delta variant (SARS-CoV-2-Taiwan-CDC#1144, B.1.617.2), which was conducted in a BSL-3 lab at Academia Sinica, Taiwan. Vero-E6 (ATCC® CRL-1586) cells were cultured in DMEM (Hyclone) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1x Penicillin-Streptomycin solution (Thermo) in a humidified atmosphere with 5% CO_2 at 37°C. The 96-well microtiter plates are seeded with 1.2×10^4 cells/100 μL/well. Plates are incubated at 37°C in a CO_2 incubator overnight. The next day tested sera were heated at 56°C for 30 min to inactivate complement, and then diluted in DMEM (supplemented with 2% FBS and 1x Penicillin/Streptomycin). Serial 2-fold dilutions of sera were carried out for the dilutions. Fifty μL of diluted sera were mixed with an equal volume of virus (100 $TCID_{50}$) and incubated at 37°C for 1 hr. After removing the overnight culture medium, 100 μL of the sera-virus mixtures were inoculated onto a confluent monolayer of Vero-E6 cells in 96-well plates in triplicate. After incubation for 4 days at 37°C with 5% CO_2, the cells were fixed with 10% formaldehyde and stained with 0.5% crystal violet staining solution at room temperature for 20 min. Individual wells were scored for CPE as having a binary outcome of 'infection' or 'no infection'. Determination of SARS-CoV-2 virus specific neutralization titer was to measure the neutralizing antibody titer against SARS-CoV-2 virus based on the principle of VNT_{50} titer (≥ 50% reduction of virus-induced cytopathic effects). Virus neutralization titer of a serum was defined as the reciprocal of the highest serum dilution at which 50% reduction in cytopathic effects are observed and results are calculated by the method of Reed and Muench.

Neutralizing antibody titers against omicron strain by pesudovirus luciferase assay: Neutralizing antibody titers were measured by neutralization assay using HEK-293T-ACE2 cells challenged with SARS-CoV-2 pseudovirus variants. The study was conducted in a BSL2 lab at RNAi core, Biomedical Translation Research Center (BioTReC), Academia Sinica. Human Embryonic Kidney (HEK-293T/17; ATCC® CRL-11268TM) cells were obtained from the American Type Culture Collection (ATCC). Cells were cultured in DMEM (Gibco) supplemented with 10% fetal bovine serum (Hyclone) and 100 U/mL of Penicillin-Streptomycin solution (Gibco), and then incubated in a humidified atmosphere with 5% CO_2 at 37°C. HEK-293T-ACE2 cells were generated by transduction of VSV-G pseudotyped lentivirus carrying human ACE2 gene. To produce SARS-CoV-2 pseudoviruses, a plasmid expressing C-terminal truncated wild-type Wuhan-Hu-1 strain SARS-CoV-2 spike protein (pcDNA3.1-nCoV-SΔ18) was co-transfected into HEK-293T/17 cells with packaging and reporter plasmids (pCMVΔ8.91, and pLAS2w.FLuc.Ppuro, respectively) (BioTReC, Academia Sinica), using TransIT-LT1 transfection reagent (Mirus Bio). Site-directed mutagenesis was used to generate the Omicron BA.1variants

by changing nucleotides from Wuhan-Hu-1 reference strain. For BA.1 variant, the mutations of spike protein are A67V, Δ69-70, T95I, G142D/Δ143-145, Δ211/L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F. Indicated plasmids were delivered into HEK-293T/17 cells by using TransITR-LT1 transfection reagent (Mirus Bio) to produce different SARS-CoV-2 pseudoviruses. At 72 hours post-transfection, cell debris was removed by centrifugation at 4,000 xg for 10 minutes, and supernatants were collected, filtered (0.45 μm, Pall Corporation) and frozen at –80°C until use. HEK-293-hACE2 cells (1×10^4 cells/well) were seeded in 96-well white isoplates and incubated for overnight. Tested sera were heated at 56°C for 30 min to inactivate complement, and diluted in medium (DMEM supplemented with 1% FBS and 100 U/ml Penicillin/Streptomycin), and then 2-fold serial dilutions were carried out for a total of 8 dilutions. The 25 μL diluted sera were mixed with an equal volume of pseudovirus (1,000 TU) and incubated at 37°C for 1 hour before adding to the plates with cells. After 1 hr incubation, the 50 μL mixture added to the plate with cells containing with 50 μL of DMEM culture medium per well at the indicated dilution factors. On the following 16 hours incubation, the culture medium was replaced with 50 μL of fresh medium (DMEM supplemented with 10% FBS and 100 U/ml Penicillin/Streptomycin). Cells were lysed at 72 hours post-infection and Relative Light Units (RLU) was measured by using Bright-GloTM Luciferase Assay System (Promega). The luciferase activity was detected by Tecan i-control (Infinite 500). The percentage of inhibition was calculated as the ratio of RLU reduction in the presence of diluted serum to the RLU value of virus only control and the calculation formula was shown below: (RLU Control-RLU Serum)/RLU Control. The 50% protective titer (NT_{50} titer) was determined by Reed and Muench method.

Inhibition of RBD (wild-type) binding to ACE2 by ELISA: The 96-well ELISA plates were coated with 2 μg/mL ACE2-ECD-Fc antigen (100 μL/well in coating buffer, 0.1 M sodium carbonate, pH 9.6) and incubated overnight (16 to 18 hr) at 4°C. Plates were washed 6 times with Wash Buffer (25-fold solution of phosphate buffered saline, pH 7.0-7.4 with 0.05% Tween 20, 250 μL/well/wash) using an Automatic Microplate Washer. Extra binding sites were blocked by 200 μL/well of blocking solution (5N HCl, Sucrose, Triton X-100, Casein, and Trizma Base). Five-fold dilutions of immune serum or a positive control (diluted in a buffered salt solution containing carrier proteins and preservatives) were mixed with a 1:100 dilution of RBD-HRP conjugate (Horse Radish Peroxidase-conjugated recombinant protein S1-RBD-His), incubated for 30 ± 2 min at 25 ± 2°C, washed and TMB substrate (3,3',5,5'-Tetra Methyl Benzidine diluted in citrate buffer containing hydrogen peroxide) is added. Reaction is stopped by stop solution (diluted sulfuric acid, H_2SO_4, solution, 1.0 M) and the absorbance of each well is read at 450 nm within 10 min using the Microplate reader (VersaMax). Calibration standards for quantitation ranged from 0.16 to 2.5 μg/mL. Samples with titer value below 0.16 μg/mL were defined as being half of the detection limit. Samples with titer exceed 2.5 μg/mL were further diluted for reanalysis.

Anti-S1-RBDWT binding IgG antibody by ELISA: The 96-well ELISA plates were coated with 2 μg/mL recombinant S1-RBDWT-His protein antigen (100 μL/well in coating buffer, 0.1 M sodium carbonate, pH 9.6) and incubated overnight (16 hours to 18 hours) at room temperature. One hundred μL/well of serially diluted serum samples (diluted from 1:20, 1:1,000, 1:10,000 and 1:100,000, total 4 dilutions) in 2 replicates were added and plates are incubated at 37°C for 1 hr. The plates were washed six times with 250 μL Wash Buffer (PBS-0.05% Tween 20, pH 7.4). Bound antibodies were detected with HRP-rProtein A/G at 37°C for 30 min, followed by six washes. Finally, 100 μL/well of TMB (3,3',5,5'-Tetra Methyl Benzidine) prepared in substrate working solution (citrate buffer containing hydrogen peroxide) was added and incubated at 37°C for 15 min in the dark, and the reaction stopped by adding 100 μL/well of H_2SO_4, 1.0 M. Sample color developed was measured on ELISA plate reader (Molecular Device, VersaMax). UBI® EIA Titer Calculation Program was used to calculate the relative titer. The anti-S1-RBD antibody level is expressed as Log_{10} of an end point dilution for a test sample (SoftMax Pro 6.5, Quadratic fitting curve, Cut-off value 0.248).

T cell responses by ELISPOT: Human Peripheral Blood Mononuclear Cells (PBMCs) were used in the detection of the T cell response. For the booster-series third-dose series extension study, ELISpot assays were performed using the human IFN-γ/IL-4 FluoroSpotPLUS kit (MABTECH). Aliquots of 250,000 PBMCs were plated into each well and stimulated, respectively, with 10 μg/mL (each stimulator) of RBD-WT+Th/CTL, Th/CTL, or Th/CTL pool without UBITh1a (CoV2 peptides), and cultured in culture medium alone as negative controls for each plate for 24 hours at 37°C with 5% CO_2. The analysis was conducted according to the manufacturer's instructions. Spot-Forming Units (SFU) per million cells was calculated by subtracting the negative control wells.

Intracellular Cytokine Staining (ICS): Intracellular Cytokine Staining and flow cytometry was used to evaluate CD4+ and $CD8^+$ T cell responses. PBMCs were stimulated, respectively, with S1-RBD-His recombinant protein plus with Th/CTL peptide pool, Th/CTL peptide pool only, CoV2 peptides, PMA+Inonmycin (as positive controls), or cultured in culture medium alone as negative controls for 6 hours at 37°C with 5% CO_2. Following stimulation, cells were washed and stained with viability dye for 20 minutes at room temperature, followed by surface stain for 20 minutes at room temperature, cell fixation and permeabilization with the BD cytofix/cytoperm kit (Catalog#554714) for 20 minutes at room temperature, and then intracellular stain for 20 minutes at room temperature. Intracellular cytokine staining of IFN-γ, IL-2 and IL-4 was used to evaluate $CD4^+$ T cell response. Intracellular cytokine staining of IFN-γ, IL-2, CD107a and Granzyme B was used to evaluate $CD8^+$ T cell responses. Upon completion of staining, cells were analyzed in a FACSCanto II flow cytometry (BD Biosciences) using BD FACSDiva software.

Statistics

For the Phase-2 extension booster vaccination study, the immunogenicity results for Geometric Mean Titer (GMT) are presented with the 95% confidence intervals. Statistical analyses were performed using SAS® Version 9.4 (SAS Institute, Cary, NC, USA) or Wilcoxon sign rank test. Spearman correlation was used to evaluate the monotonic relationship between non-normally distributed data sets. For the Phase-2 primary 2-dose series, the sample size of the trial design meets the minimum safety requirement of 3000 study participants in the vaccine group with healthy adults, as recommended by the US FDA and WHO.

US Food and Drug Administration, Emergency use authorization for vaccines to prevent COVID-19: Guidance for industry, https://downloads.regulations.gov/FDA-2020-D-1137-0019/attachment_1.pdf;

WHO Guidelines on clinical evaluation of vaccines: regulatory expectations, https://cdn.who.int/media/docs/default-source/prequal/vaccines/who-trs-1004-web-annex-9.pdf?sfvrsn=9c8f4704_2&download=true.

Results

Phase-2 booster trial population, reactogenicity and safety

Trial population: After unbinding of Phase-2 trial, 1,478 of the 3,844 healthy study participants who completed the 2-dose primary series (28 days apart) of 100 μg UB-612 (Supplementary Figure 2) were enrolled to receive an additional 100 μg booster 3rd-dose 6 to 8 months after the second shot. The booster vaccines were followed for 14 days for assessment of safety and immunogenicity. The vast majority of participants were of Taiwanese origin, with two groups aged 18-65 years (76%) and 65-85 years (24%) (Supplementary Table 1).

Reactogenicity and safety: No vaccine-related Serious Adverse Events (SAEs) were recorded; the most common solicited AEs were injection site pain and fatigue, mostly mild and transient (Supplementary Figure 3). The incidence of solicited local AEs slightly increased post-booster, mostly pain at the injection site (mild, 54%; moderate, 7%). The incidence of skin allergic reaction at post-booster was similar to post-dose 2 Fatigue/tiredness, muscle pain, and headaches that belonged to solicited systemic AEs were mostly mild. Overall, no safety concerns were identified with UB-612 (Supplementary Figures 3A-3C).

Durable T Cell responses induced by Th/CTL epitope peptides

T-cell responses to stimulation by epitope peptides (Th/CTL+RBD or Th/CTL alone) were analysed with PBMCs collected from 83 vaccinees from Immunogenicity group (n=83) on Days 57 (28 days after 2nd booster); and from 32 vaccinees from the Immunogenicity (n=18) or Safety groups (n=14) who joined the Phase-2 extension booster study to evaluate the T-cell responses in PBMCs on Days 197 to 242 (pre-boosting days) and Days 211 to 256 (14 days post-booster third dose) (Figures 2A-2F) (Table 2).

ELISpot: Vaccinees' Peripheral Blood Mononuclear Cells (PBMCs) were collected for evaluation of Interferon-γ by ELISpot. On Day 57 (28 days post-2nd dose), IFN-γ SFU (Spot Forming Unit)/10^6 cells under stimulation with RBD+Th/CTL increased from the baseline 1.0 to a high 374 SFU, which maintained strong at 261 (70%) at pre-boosting (6-8 months post-2nd dose), then rose to 444 SFU 14 days post-booster. Similar IFN-γ profiles were observed for that stimulated with Th/CTL alone, which increased from the baseline 1.3 to a high 322 SFU on Day 57, maintained at 182 SFU (~57%) at pre-boosting and remained strong at 317 SFU 14 days post-booster. Apparently, T cell responses persisted robust (60%-70% of the high peak at Day 57) long over 6-8 months.

These results indicate that UB-612 can induce a strong, durable IFN-γ$^+$ T cell immunity in the primary series and prompt a high level of memory recall upon boosting, and that the presence of Th/CTL peptides is essential and principally responsible for the bulk of the T cell responses, while S1-RBD plays only a minor role. Together with the insignificant low levels of the IL-4$^+$ ELISpot responses, UB-612 vaccination at both primary series and homologous-boosting could induce pronounced Th1-predominant immunity.

Intracellular Cytokine Staining (ICS): Along with high levels of ELISpot-based T cell responses, CD8$^+$ T cells expressing cytotoxic markers, CD107a and Granzyme B, were observed in the primary series, accounting for a remarkable 3.6% of circulating CD8$^+$ T cells after re-stimulation with S1-RBD+Th/CTL, which persisted at a substantial 1.8% upon booster vaccination. Apparently, CD8$^+$ T cell responses persisted robustly (50% of the high peak at Day 57) long over 6-8 months as well. This suggests a potential of robust cytotoxic CD8$^+$ T responses in favor of viral clearance once infection occurs.

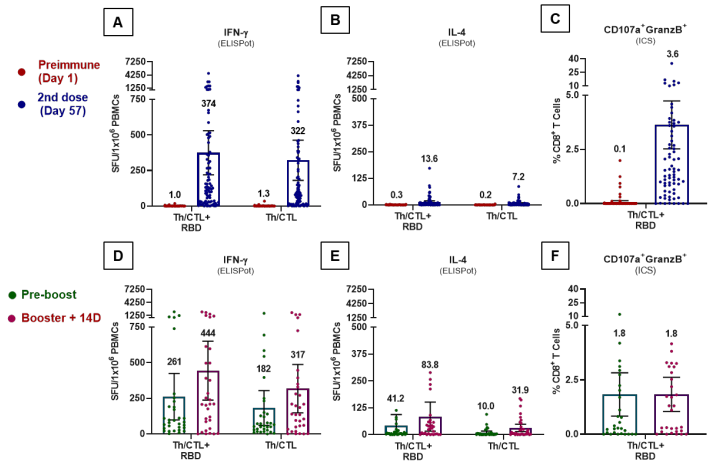

Figure 2. UB-612 induced T cell responses measured by ELISpot and ICS analyses. T-cell responses were measured by (A, D) IFN-γ and (B, E) IL-4 ELISpot at 10 ug/mL per stimulator. Spot-Forming Units (SFU) per 1 × 10^6 PBMCs producing IFN-γ and IL-4 after stimulation with the RBD+Th/CTL peptide pool or the Th/CTL peptide pool are expressed. The PBMC samples stimulated with Th/CTL+RBD were also evaluated for T cell responses by Intracellular Cytokine Staining (ICS) (C, F) by which the frequency of CD8$^+$ T cells producing the measured cytokines (CD107a and Granzyme B) in response to the stimulation of RBD+Th/CTL peptide pool are shown. Horizontal bars indicate mean with 95% CI. **Note:** (●) Pre immune (day 1); (●) Second dose (day 57); (●) Pre-boost; (●) Booster+14D.

Table 2. Summary of mean and 95% CI are presented in plots (Figure 2).

Group	Time point	n	Th/CTL+RBD Mean(95% CI)	Th/CTL Mean(95% CI)
Interferon γ	Preimmune (Day 1)	83	0.9639(0.4199-1.508)	1.289(0.3872-2.191)
	2nd Dose (Day 57)	83	374.3(219.2-529.5)	322.2(180.6-463.7)
	Pre-boost (Day 220)	32	261.46(98.62-424.3)	443.7(237.4-650.0)
	Booster (Day 234)	32	181.7(5959-303.8)	317.4(147.5-487.2)
IL-4	Preimmune (Day 1)	83	0.3133(0.1572-0.4693)	0.1928(0.05029-0.3353)
	2nd Dose (Day 57)	83	13.6(7.965-19.24)	7.253(4.436-10.07)
	Pre-boost (Day 220)	32	41.23(-11.31-93.77)	9.958(2.98-16.94)
	Booster (Day 234)	32	83.79(16.24-151.3)	31.9(14.72-49.07)
CD107a$^+$GranzB$^+$CD8$^+$	Preimmune (Day 1)	83	0.07294(0.009911-0.136)	-
	2nd Dose (Day 57)	83	3.629(2.523-4.735)	-
	Pre-boost (Day 220)	32	1.83(0.334-2.827)	-
	Booster (Day 234)	32	1.831(1.045-2.617)	-

Cross-reactive neutralizing antibodies against SARS-CoV-2 omicron and delta variants

A total of 41 serum samples (n=27 for 18-65 years; n=14 for 65-85 years) from 14 days post-booster were subjected to a pseudo virus-luciferase neutralization assay for Omicron and a live virus CPE neutralization assay for Delta variant. A total of 41 serum samples (n=27 for 18-65 years; n=14 for 65-85 years) from 14 days post-booster were subjected to a pseudovirus-luciferase neutralization assay for Omicron and a live virus CPE neutralization assay for Delta variant (Figures 3A-3D) (Table 3).

Anti-Omicron assay with pseudovirus: Based on pseudovirus neutralization assay and observed across all groups aged 18-85 years (n=41), the UB-612 booster elicited a high geometric mean 50% neutralizing titer ($pVNT_{50}$) at 6,245 against wild-type Wuhan strain (WT) versus Omicron variant at 1,196, representing a 5.2-fold reduction (Figure 3A). There was no significant age-dependent booster-induced neutralization effect between young adults (18-65 years) and the elderly (65-85 years) with respect to either anti-WT or anti-Omicron $pVNT_{50}$ level (Figure 3B). Both age groups showed a 5.0 to 6.0 fold reduction for anti-Omicron relative to anti-WT, yet the $pVNT_{50}$ of 1,196 still represents a potent viral-neutralizing strength.

Anti-Delta assay with live virus: Based on live virus neutralization assay observed across all groups aged 18-85 years (n=41), the UB-612 booster elicited a geometric mean 50% neutralizing titer (VNT_{50}) against wild-type Wuhan strain (WT) at 1,711 versus Delta variant at 1,282, representing a 1.3-fold reduction. Again, there was no significant age-dependent booster-induced neutralization effect between young adults (18-65 years) and the elderly (65-85 years) observed with respect to either anti-WT or anti-Delta VNT_{50} level (Figures 3A-3D). Both age groups showed a 1.2- to 1.7-fold reduction for anti-Delta relative to anti-WT.

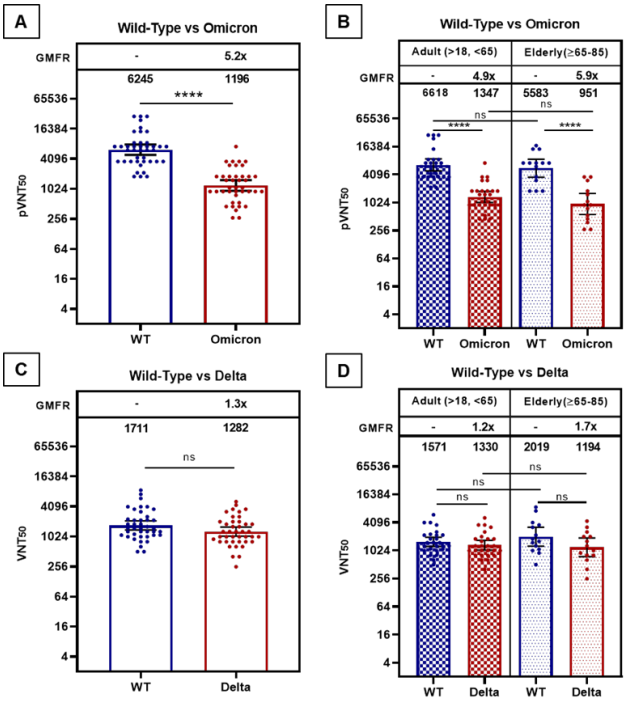

Figure 3. UB-612 booster induced potent neutralization effects against both Omicron and Delta variants. The viral-neutralizing antibody Geometric Mean Titers (GMT, 95% CI) that inhibit 50% of SARS-CoV-2 Wild-Type Wuhan strain (WT), Omicron and Delta were measured. Statistical analysis was performed by the Students t-test (ns, p.0.05; **, p.0.0001). A) The pVNT50 titers 14 days post-booster reached at 6245 against SARS-CoV-2 WT, and at 1196 against Omicron. The 5.2-fold reduction stands for 19% preservation of neutralization titers relative the Wuhan wild type. B) The pVNT50 titers are presented by two age groups. C) VNT50 titers 14 days post-booster reached at 1711 against live WT, and at 1282 against Delta. The 1.3-fold reduction stands for 75% preservation of neutralization titer relative to the WT. D) VNT_{50} titers are presented by two age groups. **Note:** ( ) Wild Type; ( ) Delta (day 1).

Table 3. Summary of Geometric Mean Titers (GMT) and 95% CI.

Assay	Age (year)	n	$pVNT_{50}$(Wild-Type) GMT(95%CI)	$pVNT_{50}$(Omicron) GMT(95%CI)	GMFR (WT/Omicron)	p value
Pseudovirus	18-85	41	6245(4926-7918)	1196(937.1-1527)	5.2	<0.0001
	18-65	27	6618(4923-8898)	1347(1027-1769)	4.9	<0.0001
	65-85	14	5583(3571-8731)	951(565.6-1599)	5.9	<0.0001
			VNT_{50}(Wild-Type) GMT(95%CI)	VNT_{50}(Delta) GMT(95%CI)	GMFR (WT/Delta)	p value
Live Virus	18-85	41	1711(1384-2116)	1282(1038-1583)	1.3	ns
	18-65	27	1571(1256-1980)	1330(1048-1689)	1.2	ns
	65-85	14	2019(1263-3228)	1194(753.4-1894)	1.7	ns

Functional correlations between ACE2:RBD (wild type) binding inhibition and viral neutralization

Of 871 participants enrolled in the Phase-2 primary 2-dose series and grouped for Immunogenicity investigation, serum samples from 87 participants who had received a booster 3rd-dose of 100 µg UB-612 were collected at Day 1 (pre-dose 1), Day 57 (28 days post-dose 2), Day 220 (pre-booster between Days 197 to 242,), Day 234 (14 days post-booster between Days 211 to Day 256). HCS from 20 SARS-CoV-2 infected individuals were also included for comparative testing by two functional assays (Figures 4A-4E) (Table 4).

Immunogenicity overview, antigenic and functional: Of the 871 Phase-2 study participants enrolled for the primary 2-dose series grouped for Immunogenicity investigation, 302 participants had their sera collected at pre-boosting and 14 days post-booster for antigenic assay by anti-S1-RBD IgG ELISA, and functional assays by ACE2:RBD binding inhibition ELISA and by neutralization against live SARS-CoV-2 wild-type Wuhan strain by cell-based CPE method. The results showed pronounced booster-enhanced increases in both target binding and inhibition/neutralization titers by respective 16 to 45 folds (Supplementary Table 2) (Supplementary Figure 4).

Potent and durable viral-neutralization and ACE2:RBD (wild type) binding inhibition: In the Phase-2 extension booster Immunogenicity group, the immune sera from 87 participants available on Day 1 (pre-dose), Day 57 (28 days post-2nd dose), Day 220 (pre-boosting, 6 to 8 months post-2nd dose), and Day 234 (14 days post-booster) were assayed for neutralization against live WT strain, which showed a high post-booster VNT_{50} titer of 738, a 17-fold increase over the pre-boosting (titer 44) and a 7-fold increase over the levels of both Day 57 (titer 10^4) and the human convalescent sera, HCS (titer 102) (Figure 4A).

A striking post-booster functional antibody-mediated inhibition of ACE2:RBD binding on ELISA was notable at a high titer (expressed in standard-calibrated antibody concentration) of 198 µg/mL, a ~57-fold increase over both the pre-boosting titer of 3.5 µg/mL and the Day 57 titer of 3.5 µg/mL, and a profound 140-fold over the HCS titer of 1.4 µg/mL. As the ELISA methodology measures neutralizing (inhibitory) antibodies against RBD binding to ACE2 receptor, the low HCS titer suggests that the majority of antibodies in HCS appears to bind more to the allosteric sites (N- or C- terminal domain of the S1) than to the orthosteric (RBD) sites.

Of note, UB-612 induces a durable neutralizing antibody titer level, observed between Day 57 (post-2nd dose) *vs.* Day 220 (pre-boosting), a 42% retainment for VNT_{50} (titer, 10^4 *vs.* 44) against live WT virus (Figures 4A-4E) and a 88% retainment for ACE2:RBDWT (µg/mL, 4.0 *vs.* 3.5) binding inhibition (Figure 4B).

The neutralization of ACE2:RBD binding on ELISA correlates well with both anti-WT and anti-Delta VNT_{50} findings, both showed a similar high correlative Spearman r=0.795 and 0.828, respectively. A lesser but significant correlation exists for ACE2:RBD binding inhibition and anti-Omicron $pVNT_{50}$, with a Spearman r=0.565. Of 871 participants enrolled in the Phase-2 primary 2-dose series and grouped for Immunogenicity investigation, serum samples from 87 participants who had received a booster 3rd-dose of 100 µg UB-612 were collected at Day 1 (pre-dose 1), Day 57 (28 days post-dose 2), Day 220 (pre-booster between Days 197 to 242,), Day 234 (14 days post-booster between Days 211 to Day 256). HCS from 20 SARS-CoV-2 infected individuals were also included for comparative testing by two functional assays (Figures 4C and 4E).

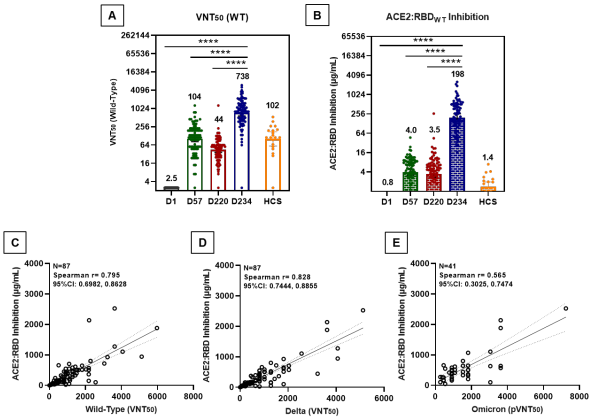

Figure 4. Functional correlations between ACE2:RBD (wild type) binding inhibition and viral-neutralization. A) viral-neutralizing titer (VNT_{50}) against live wild-type Wuhan strain (WT) by CPE method; B) the antibody concentration calibrated with an internal standard for ACE2:RBD binding inhibition by ELISA. The correlations are explored between the two function assays, i.e., ACE2:RBD binding inhibition ELISA and the viral-neutralizing titers against the live virus (VNT_{50} for original wild-type and Delta strains) or the psuedovirus ($pVNT_{50}$ for Omicron strain). The RBDWT stands for the RBD binding protein domain bearing amino acid sequence of the original SARS-CoV-2 Wild-Type (WT) Wuhan strain. The correlations were explored for; C) ACE2:RBD inhibition *vs.* anti-WT VNT_{50}; D) ACE2:RBD inhibition *vs.* anti-Delta VNT_{50}; E) ACE2:RBD inhibition *vs.* anti-Omicron $pVNT_{50}$. The correlation coefficients were evaluated by Spearman r with 95% CI. Statistical analysis was performed with the Student's t-test (ns, p>0.05; ***, p<0.001; ****, p<0.0001).

Table 4. Summary of Geometric Mean Titer (GMT) and 95% CI are presented for plots plots as shown in Figures 4A and 4B.

Assay	Time Point	n	VNT_{50}(Wild-Type) GMT(95%CI)	GMFI (of Booster)	p value
VNT_{50}	Preimmune (D1)	87	2.5(2.5-2.5)	295	<0.0001
	2nd Dose (D1)	87	104.4(82.32-132.4)	7	<0.0001
	Pre-boost (D220)	87	44.46(36.78-53.75)	17	<0.0001
	Booster (D234)	87	737.8(582.2-934.9)	1	-
			ACE2:RBD Inhibition(µg/mL] GMT (95%CI)	**GMFI (of Booster)**	**p value**
ACE2:RBD	Preimmune (D1)	87	0.8(0.8-0.8)	247	<0.0001
	2nd Dose (D1)	87	3.953(3.169-4.93)	50	<0.0001
	Pre-boost (D220)	87	3.468(2.701-4.453)	57	<0.0001
	Booster (D234)	87	197.7(151.7-257.6)	1	-

Discussion

The present Phase-2 extension study (participants aged 18-85 years) has shown that UB-612 booster (the third dose) is safe and well tolerated, and can induce unusually high levels of cross-reactive neutralizing titers against the Delta and Omicron variants that parallel with robust, cross-reactive VoC antigen-specific T cell immunity. In particular, the booster can induce essentially the same striking degree of neutralizing strength against Delta or Omicron for both the elderly and the young adults; and the high viral-neutralizing titers bear only a modest ~1.0-fold (anti-Delta) to 5.0-fold (anti-Omicron) GMFR relative to WT virus (Figure 3). Similar fold-reduction landscape is obvious in the primary and booster series in the Phase-1 trial (participants aged 20-55 years), across all VoCs and other Variants of Interest (Supplementary Figures 5 and 6).

On safety, no concerns of vaccine-related SAE were identified (with ~4000 individuals vaccinated so far). While UB-612 has not yet been deployed geographically wide enough, its peptide-protein subunit composition containing aluminum and CpG as the adjuvants suggests that it should have a high safety profile, unlike mRNA and adeno-vectored vaccines that, upon repeat dosing, could have more severe adverse events including rare but serious adverse reactions such as myocarditis, pericarditis, Guillain-Barre syndrome, and thrombosis-thrombocytopenia [25].

On cellular immunity, UB-612 can induce robust and long-lasting Th1 cell responses and prompt a high level of memory recall upon boosting. In the Phase-1 primary series, the post-2nd dose PBMCs stimulated RBD/Th/CTL can induce high antigen-specific Th1-dominant IFN-γ^{+}-ELISpot T cell responses at 254 SFU/10^{6} cells and a long-lasting response level with ~50% retained (the pre-boosting 121 *vs.* peak 254) >6 months post-2nd dose [24]. In the Phase-2 primary series, a higher SFU units of 374 post-2nd dose and of 261 >6 months later (pre-boosting) represents even a higher 70% (261 *vs.* 374) retainment of T cell immunity, with the SFU surged to 444 post-booster. The minor Th2 IL-4^{+}-T cell response found in the Phase-2 trial reconfirms that observed in the Phase-1 study [24].

Evidently, the Th/CTL peptide pool (containing N, M, and S2 epitope peptides) is the principal driver of T cell immunity, with SFU units at 322 post-2nd dose and maintained at 317 post-booster (Figures 2A and 2D). Furthermore, a strong, durable cytotoxic (CD107a^{+}-Granzyme B^{+}) activity of CD8^{+} T Lymphocytes (CTL) accounting for a high CTL frequency at 3.6% post-2nd dose and persists at 1.8% pre-booster (Figures 2C and 2F) indicates a 50% retainment of CTL activity more than 6 months post-2nd dose, which maintains at the same level observed 14 days post-booster.

The magnitude of the UB-612 booster-recalled T cell immunity in the present Phase-2 homologous-boosting (SFU units range ~320-450) was found to be greater than any of those produced under "mix-and-match" heterologous-boosting settings by the currently authorized mRNA, adeno-vectored, and Spike protein-based vaccines, which reported SFU/10^{6} PBMC units (stimulated with spike peptide-specific to WT, Delta and Beta) to be in the range of 40 to 150 [26]. That the rationally-designed UB-612 S1-RBD-sFc protein-subunit vaccine is armed with T cell immunity-promoting N (Nucleocapsid), M (Membrane) and S2 (Spike) epitope peptides (Table 1), which are highly conserved across all VoCs, may underlie the UB-612's differentially pronounced booster-induced T cell immunity.

The much more booster-enhanced T-cell immunity *via* UB-612 is also supported by the development of a plain T-cell vaccine containing a six-peptide backbone that, as a T-cell booster, triggered dramatic multifunctional CD4 and CD8 T-cell responses [27], which showed notable benefits to B-cell deficient, immune compromised patients who could not mount B-cell antibody responses. This raises the concerns over the fact that humoral antibody response has long been used as a sole metric of protective immunity [28,29], which lacks full understanding of human post-vaccination immune responses as antibody response is generally shorter-lived than virus-reactive T cells [30-32].

Further, the SARS-CoV-2's non-spike structure proteins of Envelope (E), Membrane (M) and Nucleocapsid (N) are the regions critically involved in the host cell interferon response and T-cell memory [19-22]. These structural proteins of virus' main body when presented would fall beyond recognition by the currently authorized vaccines that based on the outer spike only, implicating that these vaccines bear an intrinsic shortfall, lacking at least N and M-specific T cell immunity [33]. The adaptive immune response is a major determinant of the clinical outcome, to which T cell immunity plays a central role in the control of SARS-CoV-2 infection and its importance have been underestimated thus far [34]. In fact, the non-spike viral structure proteins have long been utterly overlooked since the development of COVID vaccines.

On humoral immunity, UB-612 booster shots in both Phase-1 and Phase-2 studies exhibit similar high-titer profiles. The Phase-1 post-2nd dose booster (adults aged 20-55 years) induced unusually high cross-neutralizing anti-Delta VNT_{50} titers against live viruses (WT 3,992 *vs.* Delta at 2,358; 1.7-fold reduction) (Supplementary 6A) and the Phase-2 booster vaccination (adults aged 18-85 years) elicited pronounced VNT_{50} titers as well (WT at 1,711 *vs.* Delta at 1,282; 1.3-fold reduction) (Figure 3C). In addition, although not investigated in the Phase-2 study, we have found in the phase-1 primary series that UB-612 could induce a long-lasting viral-neutralizing antibody against WT strain with a half-life of 187 days [24].

Potent post-booster and anti-Omicron effects ($pVNT_{50}$) against pseudo virus are notably similar. UB-612 neutralizes WT at 12,778 *vs.* Omicron at 2,325 with 5.4 fold reduction in Phase-1 (Supplementary 6B); and WT 6,245 *vs.* 1196 with 5.2-fold reduction in Phase-2 (Figure 3A). Though with a ~5-fold reduction for Omicron in both clinical studies, the neutralizing $pVNT_{50}$ in the range of 1,196 to 2,325 are of high potency. Comparatively, the differential fold reductions reveal that UB-612 may neutralize Delta with a potency around 3-4 fold greater than against Omicron.

Table 5. Viral-neutralizing antibody titers against SARS-CoV-2 Wild-Type (WT) and Omicron variant upon homologous boosting.

Vabbines[a] Homo-booster	Booster time (post-2nd dose)	NeuAb assay (Method/Unit)	WT (GMT)[b]	Omicron (GMT)[c]	WT/Omicron (GMFR)[d]
UB-612	6-8 months	PNA/$pVNT_{50}$	6245	1196	5.2
mRNA-1273	9 months	PNA/ID_{50}	4216	650	6.5
BNT162b2	>6 months	PRNT/$PRNT_{50}$	368	164	2.2
MVC-COV1901	6 months	PNA/ID_{50}	1280-640	160-80	8.7
AZD1222	6 months	FRNT/$FRNT_{50}$	726	57	12.7
BBIBP-CorV	8-9 months	PNA /$pVNT_{50}$	295	15	20.1

Note: [a] Vaccines reported of post-booster GMT for mRNA-1273 [35], BNT162b2 [36], MVC-COV1901 [37], AZD1222 [38], BBIBP-CorV [39], and the UB-612 in the present report (Figure 3A);
[b] GMTs against WT measured at 14 or 28 days post-booster third dose;
[c] Source of Omicron Strain for assay: FRNT/live clinical isolate; PNA/pseudovirus; PRNT recombinantly engineered with Omicron spike;
[d] GMFR against WT/Omicron strain of SARS-CoV-2
Abbreviation: PNA: Pseudotyped virus Neutralization Assay; PRNT: Plaque Reduction Neutralization Test; FRNT: Focus Reduction Neutralization Test; GMT: Geometric Mean Titer; GMFR: Geometric Mean Fold Reduction; WT: Wild Type strain of SARS-CoV-2; Omicron: The Omicron variant of the SARS-CoV-2 WT.

Furthermore, there have been reports on homologous booster vaccination by other vaccine platforms (Table 5) [35-39] where the post-booster anti-WT $pVNT_{50}$ were reported to range from the low 295 to the high 6,245 (UB-612), and the anti-Omicron to range from the low 15 to the high 1,196 (UB-612). The UB-612's anti-Omicron $pVNT_{50}$ profile in contrast to other vaccine platforms is in good agreement with the finding of high performance of the UB-612 booster combating live WT and live Delta virus strains observed in the Phase-1 booster vaccination [24]. In contrast to other vaccine platforms, anti-WT VNT_{50} titers were reported to range from the low 122 to the high 3,992 (UB-612), and the anti-Delta to range from the low 54 to the high 2,358 (UB-612). UB-612 appears to bear a competitive edge over other vaccine platforms in neutralizing both Omicron and Delta variants.

We also observed significant $pVNT_{50}$ titers against pseudovirus of SARS-CoV-2 variants for the Phase-1 post-2nd dose serum (28 days post-2nd dose), which ranges from 76.6 for Beta, 224 for Gamma, 246 for Delta, 374 for Alpha, and 394 for WT in the Supplementary Figure 5, representing an 1.0 to 5.1-fold reduction relative to the WT, suggestive that UB-612 could be effective against all VoCs.

The profound post-booster neutralization effect against both live WT and live Delta, and pseudovirus WT and Omicron variants, illustrates one unique feature of UB-61 that is the immune response is directed solely at the receptor binding domain (RBD) that reacts with ACE2 receptor. The RBD-focused design leaves little non-conserved sites on the spike for viral mutation, thus boosting promptly recalls of high levels of both viral-neutralizing and RBD-ACE2 binding inhibition antibodies and both functional activities are significantly inter-correlated. Of note, in this Phase-2 primary series, the anti-WT VNT_{50} and the ACE2: RBD binding inhibition titer is durable over the Day 57 and Day 220.

The finding that the UB-612 induced much higher fold-increases (GMRIs) in blocking the RBD-ACE2 interaction than that by HCS (Figure 4B) suggests that most of the antibodies in HCS may bind allosterically to the viral spike (N-or C- terminal domain of the S), rather than orthosterically to the RBD sites and may include some unwanted enhancing antibodies. This warrants further investigation including sera from re-infections and breakthrough infections from all vaccine platforms.

Booster vaccination can reduce rates of hospitalization and severe diseases, yet offers less protection against infection and mild disease [1-11]. The pathologically-lesser but hyper-transmissible Omicron shall not be treated as a trivia as the coming of flu. COVID by itself can take a serious long-term toll on heart health [12], presumably to stay as a part of long-haul COVID [13]. This toll, beyond the already known myocarditis and pericarditis associated with mRNA vaccines, encompasses a cluster of inflammatory cardiovascular disorders that elevates, depending on COVID severity, from asymptomatic, symptomatic, to acute infection cases [12,15,16]. Facing ever-emergent variants and long-haul COVID, urgent development of new vaccines that can enhance immunity with sufficient magnitude, durability and breadth of virus coverage has been strongly advocated [17,18].

As memory B and T cells are critical in secondary responses to infection, a successful vaccine must generate and maintain immunological memory and to mount a rapid recall of effective humoral and cellular responses upon natural exposure or vaccine boosting [40,41]. By rational design, UB-612 vaccine product has demonstrated such important vaccine features of balanced B- and T-immunity through these clinical studies.

In particular, the UB-612-induced T cell immunity would comprehensively recognize Spike (S2) and non-spike structure N (Nucleocapsid) and M (Membrane) proteins, which may boost the potential in favor of viral clearance of the infected cells regardless of mutations found in Delta and Omicron, as their mutation sites are not to overlap any of the amino acid residues on the precision-designed S2, N, and M epitope peptides that are highly conserved (or rarely mutate) across all VoCs [23] (Table 1). Presumably, the viral proteins on Omicron that are substantially conserved can serve as strong T-cell activators and induce long-lasting T-cell response [42], which can synergize with B-cell memory for enhanced immunity. And, as non-spike structure N and M proteins fall beyond recognition by the currently authorized COVID vaccines, UB-612 may serve as a universal vaccine to fend off new Variants of Concern such as Delta, Omicron and other ever-emergent SARS-CoV-2 variants with broad, durable and balanced B- and T-cell immunity.

Conclusion

The UB-612-induced T cells would comprehensively recognize Spike (S2) and non-spike structure N (Nucleocapsid) and M (Membrane) proteins that may seed the potential favorable for viral clearance once infection occurs, regardless of mutations in the spike or non-spike protein domains on Delta and Omicron. As non-spike structure N and M proteins fall beyond recognition by the currently authorized COVID vaccines, UB-612 may serve as a universal vaccine to ward off VoCs including Delta, Omicron and ever-emergent SARS-CoV-2 variants with a broad, durable and balanced B-and T-cell immunity.

Limitation

This study has four limitations. First, UB-612 has not yet been widely deployed geographically. Second, in-depth biomarker analysis beyond those described in this report was not conducted due to insufficient volume of blood and samples retained. Third, we used Th/CTL peptide pool for an overall *in vitro* assessment of immune enhancement upon booster without delineation as to which peptide contributes most to mounting T-cell immunity in each of the vaccinees, though pooling may produce synergistic effect. Fourth, the post-booster functional analyses were carried out for a short period of time, which lacks information as to how durable the B- and T-cell immune response would last. However, it is worthy to note that UB-612 multitope subunit vaccine is the first designer vaccine to elicit a potent balanced B and T-cell immunity against SARS-CoV-2 infection. Development of a new generation of such B-T combined vaccines has been explored.

Author Contributions

CYW, KPH, YHS and WJP, were responsible for vaccine development including vaccine formulation design, protocol design and implementation of the clinical studies, acquisition and interpretation of the clinical data. CYW, WJP, BSK and YHS had full access to and verified all the data in the study and take responsibility for the integrity and accuracy of the data analysis. BSK and CYW drafted and prepared the manuscript. CCK provided critical review of the entire scope of the manuscript. All authors reviewed and approved the final version of the manuscript. CYW had final responsibility for the decision to submit for publication.

Data Sharing

The study protocols are provided in the Supplemental Appendices. Individual participant data will be made available when the trial is complete with data to be shared through a secure online platform.

Declaration of Interests

CYW is co-founder and board member of UBI, United BioPharma, and UBI Asia, and named as an inventor on a patent application covering the composition of matter of this SARS-CoV-2 vaccine (Wang Chang Yi, et al. Designer Peptides and Proteins for the detection, prevention and treatment of Coronavirus Disease, 2019 (COVID-19). WO2021/168305A1. International Publication date August 26th, 2021. WJP is named as a co-inventor on the same patent application covering this SARS-CoV-2 vaccine. CYW, WJP and BSK are employees within the UBI group.

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