Potent Anti-Delta Effect By a Booster Third-Dose of UB-612, a Precision-Designed SARS-CoV-2 Multitope Protein-Peptide Vaccine

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Abstract

SARS-CoV-2 breakthrough infection occurs due to waning immunity time-to-vaccine, to which the globally-dominant, highly-contagious Delta variant is behind the scene. In the primary 2-dose and booster series of clinical Phase-1 trial, UB-612 vaccine, which contains S1-RBD and synthetic Th/CTL peptide pool for activation of humoral and T-cell immunity, induces substantial, prolonged viral-neutralizing antibodies that goes parallel with a long-lasting T-cell immunity; and a booster (3rd) dose can prompt recall of memory immunity to induce profound, striking antibodies with the highest level of 50% viral-neutralizing GMT titers against live Delta variant reported for any vaccine. The unique design of S1-RBD only plus multitope T-cell peptides may have underpinned UB-612's potent anti-Delta effect, while the other full S protein-based vaccines are affected additionally by mutations in the N-terminal domain sequence which contains additional neutralizing epitopes. UB-612, safe and well-tolerated, could be effective for boosting other vaccine platforms that have shown modest homologous boosting.

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Introduction

The COVID-19 pandemic continues to cost human lives and sap the world's economy and healthcare system with a sizable pool of asymptomatic contagious coverts[1,2] that sustain transmission, and a breakthrough infection that occurs under complete 2-dose vaccination due to waning immunity time-to-vaccine. Amongst the globally-dominant Variants of Concern (VoCs), the Delta variant behaves as contagious as chickenpox, and those infected can carry up to 1,000 times more virus in their nasal passages than other variants[3].

People fully-vaccinated with authorized COVID-19 vaccines can develop breakthrough cases, carry as much of the virus as unvaccinated people, and contribute to spread of the virus worldwide[4,5]. To maintain the vaccine protection against the Delta variant, many regulatory agencies have approved or are considering a booster not only for the elderly and the high-risk populations, but also for healthy individuals amid debates[6–11].

While neutralizing antibody levels correlate with vaccines' protection efficacy[12,13], substantial activation of $CD4^+$ and $CD8^+$ T cells by viral antigens are also critical for better duration of robust immunity and immunological memory[14,15]. Early induction of functional SARS-CoV-2-specific T cells is critical with rapid viral clearance and amelioration of disease[16]. Thus, T cell responses elicited by peptides representing viral structural and non-structural proteins are of increasing interest in the control of infection[17], an immunological-base by which UB-612 is designed for clinical development.

UB-612 vaccine has a unique composition of matter[18] (**Supplementary Fig. 1**), containing (a) a B cell immunogen as SARS-CoV-2 S1-receptor binding domain (RBD) protein, constrained to preserve its ACE2 binding site, as a single chain Fc fusion protein; and (b) T cell immunogens as designer synthetic

peptides, incorporating helper T-cell (Th) and cytotoxic T-cell (CTL) epitopes from the conserved regions of the membrane (M) proteins, nucleocapsid (N) and spike S2 proteins among available viral variants, to activate both B and T- cell immunities.

This multitope composition represents different viral T epitopes known to bind to multiple Class I and Class II Major Histocompatibility Complexes (MHC-I and MHC-II)[19,20], by which UB-612 is unique and distinct from other full length spike protein-based vaccines.

Here we report the interim 14-day results of UB-612 from the extension arm of a 196-day phase-1 trial, where a booster (3rd) dose of 100 μg was administered to 50 healthy adults who had originally received 10-, 30-, and 100-μg in a 2-dose primary series. The booster dose induced the highest level of viral-neutralizing titers against the Delta variant reported for any vaccine. UB-612 is safe and well tolerated, and has induced long-lasting high neutralizing antibody titers and durable antigen-specific T-cell responses.

Results

Trial population

The booster vaccination was an 84-day extension from the 196-day open-label phase-1 study involving 60 healthy adults, aged 20-55 years (**Fig. 1; Table 1**). In the primary series, the participants in three dose groups (n = 20 each) received two doses (28 day-apart) of UB-612 at 10, 30, or 100-μg. Following the primary series, 50 participants were enrolled to receive additional one 100-μg booster over 6 months after the second shot, for the 10-μg (n = 17), 30-μg (n = 15), and 100-μg (n = 18) dose groups. The boosted participants were followed up for 14 days for interim evaluation on safety and immunogenicity, and to be monitored until 84 days post-booster.

Reactogenicity and safety

In the 196-day primary series and up to 14 days post-booster, neither severe adverse effects (SAE, grades 3 and 4) nor dose-limited increase in incidence or severity were recorded. The solicited local and systemic AEs reported within 7 days in all vaccination groups (**Fig. 2**) were mild (grade 1) and transient, with lower frequencies for most systematic reactions than local reactions. The incidences of solicited local AE were comparable after the first and second vaccination and slightly increased after the booster dose (**Fig. 2a**), of which the most common post-booster solicited local AE was pain at the injection site (60-71%). The incidence of solicited systemic AEs were similar after each vaccination (**Fig. 2b**), of which the most common post-booster solicited systemic AE was fatigue (11-33%). The safety profiles observed in the primary 2-dose vaccination series and the booster phase were similar.

Neutralizing antibodies against live SARS-CoV-2 wild-type and Delta variant

A booster 100-mg dose induced robust neutralizing antibody against live SARS-CoV-2 wild type (WT, Wuhan strain) and live Delta variant in all participants, i.e., with an 100% responder rate (**Fig. 3**)**.** In the 10-, 30- and 100-μg UB-612 dose groups, the booster elicited 50% viral-neutralizing GMT titters (VNT50) against WT of 4643, 3698 and 3992 in the three dose groups, respectively (**Fig. 3a-3c; Supplementary Table 1**), representing an 104-, 118- and 37-fold respective increase over the peak responses in primary series (14 days post-dose 2, i.e., Day 42), and a 465-, 216- and 65-fold respective increase over the pre-boosting levels. In parallel to VNT50, the anti-WT profiles corresponding to the International Unit (IU/mL) were notably similar (**Supplementary Fig. 2**).

The booster dose induced strikingly high VNT_{50} titers against Delta variant as well, reaching at 2854, 1646 and 2358 (**Fig. 4a**), which represent a modest 1.6-, 2.4-, and 1.7-fold respective reduction (i.e., a preservation of ~63%, ~42% and ~59% neutralizing strength) for the 10-, 30- and 100-μg groups, relative to WT.

The neutralizing antibodies in the primary series were found to be long-lasting for the 100-μg group, with highest increases in VNT50 against WT observed at 14 to 28 days post-dose 2, as compared to the lower 10- and 30-mg groups (**Figs. 3a-3c**). Of which, the highest neutralizing titters (108 at Day 42; 103 at Day 56) (**Fig. 3c**) were similar to that of control human convalescent serum (HCS) (102). Prior to boosting (Days 255 to 316), none of the 18 participants (0%) in the 100-μg group with VNT50 titters falling below the assay limit (LLOQ), suggesting that the induced neutralizing effect could persist for a long period of time.

Using the exponential model fitting over Days 42 to 196 for the 100-mg group (the dose level investigated in the Phase-2 trial), the neutralizing antibody VNT50 and IU/mL GMT slowly declined with a half-life of 187 days (**Fig. 4b**) and 182 days (**Fig. 4c**), respectively. Assuming the slow decay kinetics applied to the booster vaccination, the VNT50 values of 3992 against WT and 2358 against Delta after boosting are predicted to decline to 1054 (278) and 622 (164) on Day 360 (720), respectively (**Fig. 4d**). These observations suggest of a potential for a single UB-612 booster dose to produce neutralizing antibodies capable of preventing illness caused by Delta and other variants for 24 months or longer.

Antigenic anti-S1-RBD antibody responses

ELISA results of the antigenic S1-RBD antibody binding (**Figs. 5a-5c**), largely consistent with the anti-WT VNT50 data (**Figs. 3a-3c**), showed the 100-mg vaccinated group produced the highest immune responses over the 196-day primary series (**Fig. 5c**), with GMT of 2240 at Day 42, which far exceeded the 141 from the 20 human convalescent sera (HCS). Upon booster vaccination, the anti-S1-RBD GMT titers in the three dose groups topped at 7154 to 9863 (3- to 28-fold increase over the peaks at primary series); similarly, profound 37- to 378-fold increases were observed as compared to the pre-boosting levels (**Figs. 5a-5c**).

In the period of both primary and booster series, the S1-RBD ELISA results correlated well with VNT50 findings (Spearman r = 0.9073) (**Figs. 5d)**. Of note, good correlation existed also between the anti-

S1-RBD antibody titers and the WHO International Reference-based Binding Antibodies Unit (BAU/mL); and similar boosting patterns were notable in terms of BAU (**Supplementary Fig. 3**).

Functional inhibition (neutralization) of S1-RBD binding to ACE2

ELISA results of the functional inhibition (neutralization) against the S1-RBD:ACE2 interaction (**Figs. 6a-6c**), largely consistent with the anti-WT VNT50 data (**Figs. 3a-3c**), showed again the 100-mg dose group produced the highest immune responses over the 196-day primary series (**Fig. 6c**), the anti-S1-RBD:ACE2 qNeuAb level of 6.4 mg/mL at Day 112, which far exceeded the 1.4 mg/mL from the 20 human convalescent sera (HCS). Upon booster vaccination, the anti-S1-RBD:ACE2 qNeuAb levels reached at 303 to 521 mg/mL (77 to 168 fold-increase over the peaks at primary series); similarly, profound 82- to 579-fold increases were observed as compared to the pre-boosting levels (**Figs. 6a-6c**).

Similar to the good relationship of anti-S1-RBD antibody by ELISA vs. VNT50 by CPE assay (**Fig. 5d**), in the period of both primary and booster series, the results of S1-RBD:ACE2 binding inhibition correlated well with VNT50 findings (Spearman r = 0.9012) (**Figs. 6d)**. Thus, both S1-RBD binding and S1-RBD:ACE2 binding inhibition on ELISA corroborate the results of anti-WT VNT50 by CPE assay (**Figs. 3a-3c**), i.e., UB-612 booster can elicit profound immune responses in vaccinated subjects regardless how low their pre-boosting levels are.

It is important to point out that across the dose groups, “functional antibodies” measured by inhibition of the S1-RBD:ACE2 interaction reached at 303 to 521 mg/mL (**Figs. 6a-6c**), which topped 216- to 372-fold over that (1.4 mg/mL, below the assay limit) of the control human convalescent sera (HCS). This suggests that the majority of antibodies in HCS appears to be less-inhibitory against S1-RBD:ACE2 interaction than the allosteric sites causing neutralization effect measured by the CPE assay.

Analyses of T-cell responses by ELISpot and Intracellular Cytokine Staining

In primary vaccination series, peripheral blood mononuclear cells (PBMCs) were collected from vaccinees for evaluation by ELISpot of T cell responses. The highest antigen-specific spot forming unit (SFU)/10^6 PBMC responses were observed with the 100-μg dose group: estimated to be 254 by stimulation with S1-RBD+Th/CTL peptide pool and 173 by Th/CTL peptide pool (**Fig. 7**), demonstrating that the Th/CTL peptides in the vaccine were essential and principally responsible for T cell responses.

On Day 196, the Interferon-g$^+$-T cell responses for the 100-μg dose group remained at levels of ~50% from the peak T-cell responses, which descend from 254 to 121 with RBD+Th/CTL peptide pool restimulation, or from 173 to 86.8 with Th/CTL peptide restimulation only (**Fig. 7**). This observation suggests that UB-612 vaccine elicited T cell responses after two shots were long-lasting as well that persist for at least 6 months.

Using a modified ELISpot method, preliminary analysis on vaccine-induced memory T cell response Day-14 post-booster vs. pre-boosting showed a modest elevation in IFN-g SFU/10^6 PBMCs (**Supplementary**

Fig. 5a). The trend of increases from 19.9 to 68.1, 101 to 144 and 31.3 to 43.1 for the 10-, 30- and 100-μg dose groups, respectively, in response to the restimulation of S1-RBD+Th/CTL peptide pool, indicating substantial antigen-specific memory T cells were retained even at more than 6 months after the primary vaccination series and these T cells were further boosted by a subsequent vaccination. In contrast with the IFN-g, the IL-4 responses were far lower in all three dose groups (**Supplementary Fig. 5b**), suggesting the induction of a Th1-predominant cellular response by UB-612 vaccination.

Substantial antigen-specific $CD4^+$ T cells producing Th1 cytokines (IFN-γ and IL-2) were observed in PBMCs collected prior to the booster dose after restimulation with different pools of vaccine antigens in all three dose groups, while only minimal Th2 cytokine (IL-4)-producing cells were detected (**Supplementary 6a**). Remarkably, robust antigen-specific $CD8^+$ T cells expressing cytotoxic markers CD107a and Granzyme B were detected after two shots of UB-612 in participants prior to receiving booster dose (**Supplementary 6b**). The results, consistent with that from ELISpot, showed a durable memory T cell response which was Th1 prone in both helper $CD4^+$ and cytotoxic $CD8^+$ T cell responses assessed by ICS and flow cytometry.

Discussion

As waning immunity after a complete 2-dose vaccination has been associated with breakthrough infections with Delta variant, recommendations for a booster dose at 6 months after the primary series have been implemented or are under consideration[6-11,21,22]. SARS-CoV-2 will continue to evolve and new variants may lead to immune escape resulting in reduced effectiveness of current vaccines[23].

New vaccines tailored to Delta variant have been thought over[24] as Delta is much less sensitive than WT to the vaccine-induced antibodies, more resistant to antibodies generated from natural SARS-CoV-2 infection, and is so contagious as to bring about breakthrough infection[25]. Thus, in the absence of a Delta-specific vaccine, a booster with the currently available vaccines may be needed, in addition to continued public health measures, such as face masking and social distancing.

There have been a number of reports on booster vaccination revealing by some but not all vaccine platforms[26-31]. Though it is difficult to directly compare neutralizing antibody titers induced by booster vaccination among distinct platforms due to heterogeneity of neutralization assays, the immune memory against prototype virus could be demonstrated by comparing the fold-increases in neutralizing antibody titers of the peak responses after primary versus booster vaccination series.

In regard of the booster effect against WT (**Table 2**), the 50% viral-neutralizing GMTs or the like, measured at 14 or 28 days post-booster, were shown to range from 143 to 6039, with the fold increases (GMFIs) ranging from 1.7 to 37. UB-612 vaccine in the 100-mg dose group shows the anti-WT VNT_{50} to be 3992, representing a 37-fold increase over the primary peak response of 108 (**Fig. 3c**).

The post-booster 50% viral-neutralizing GMTs against the Delta variant have been reported to range from 206 to 2358. Compared to WT, with the fold reductions (GMFRs) ranging from 1.2, to 3.6 (**Table 2**). After boosting, UB-612 elicited a strikingly high anti-Delta neutralizing VNT_{50} titer of 2358 (**Fig. 4a**), which preserves a ~60% neutralizing strength relative to the anti-WT VNT_{50} titer of 3992, i.e., with a modest 1.7-fold reduction.

With such an unusually high anti-WT neutralizing fold-increase, supported by the T helper immunity elicited by UB-612 during the primary vaccination, UB-612 appears to be a potent booster that can prompt recall of high levels of neutralizing antibodies. Since heterologous boosting may be more efficient at stimulating high antibody responses than homologous boosting[32], we suggest that UB-612 could be effective for boosting other vaccine platforms, particularly adenovirus vectored and inactivated vaccines that have shown modest homologous boosting.

The UB-612's post-booster preservation of substantial anti-Delta neutralizing activity is consistent with that observed in the primary series of the phase-1 trial, where UB-612 retained a remarkable 83% (1.2-fold reduction) based on the Delta-vs.-WT VNT_{50} of 212-vs.-255 (**Supplementary Fig. 4**). UB-612 also preserved notable neutralizing antibodies against the Alpha B.1.1.7 with 62% retained (1.6-fold reduction), Gamma P.1 with 42% retained (2.4-fold reduction), while that against Beta B.1.351 was weaker, with 23% retained (4.3-fold reduction) (**Supplementary Fig. 4**). By comparison, in a reported primary vaccination series, BNT162b2 mRNA vaccine showed a 5.8-fold reduction against the Delta variant relative to the wild type[33], significantly more reduced than against B.1.1.7 (2·6-fold), and on a similar order to the reduction observed against B.1.351 (4·9-fold).

These viral-neutralizing data suggest a potential for UB-612 to offer a broader coverage of SARS-CoV-2 variants. We propose that this finding may be due to fact that the vaccine contains only the S1-RBD instead of the full-length S protein. Thus, the neutralizing antibody response is focused on the functionally most important component of the virus.

The Delta variant (B.1.617.2) contains at least ten mutations in the S protein[34]. UB-612 is composed only of the RBD with only two mutations (L452R) (T478K) that would influence neutralization[35]. Of which, however, only T478K is critical and much more influential than L452R, as the latter is an epitope for some, but not most, neutralizing antibodies[36,37], while the former is unique to the Delta variant[38] and is located in the ACE2 binding site mainly involving in increased ACE2 binding affinity[39]. The T478K mutation is close to the E484K mutation that facilitates antibody escape[40, 41].

Relative to the S1-RBD design in UB-612 vaccine, the other full S protein-based vaccines are affected additionally by mutations in the N-terminal domain sequence which contains additional neutralizing epitopes. Overall, the differences in antigenic composition could account for the observations that UB-612 vaccination preserves substantial neutralizing antibodies by 60-80% against Delta strain (**Fig. 4a; Supplementary Fig. 4**).

Further, a booster dose may offer a chance of bridging the fold-reduction gap of WT-vs.-Delta as shown with the BNT162b2, 5.8-fold in primary series[33] and 1.2-fold upon boosting[29]. Importantly, a study in Israel supported that a booster with BNT162b2, in fact, has significantly improved protection from infection and serious illness among people aged 60 and older[42].

In addition, UB-612 has demonstrated induction of a durable neutralizing antibody response with a long half-life of 187 days revealed in the primary series (**Fig. 4b**), doubling that by mRNA-1273 (90 days)[43]. The long-lasting nature of B-humoral and T-cellular immune responses of UB-612 (100-mg dose group) (**Fig. 7**) could be of an advantage when vaccine's short durability has become a growing concern[6,44,45] over the breakthrough infection[4-6]. The cause of breakthrough infection has been the waning immunity time-to-vaccine[46,47].

Virus-specific humoral and cellular immunity act synergistically to protect the host from viral infection. Using humoral antibody response as a sole metric of protective immunity lacks full understanding of human post-vaccination immune responses, as antibody response is shorter-lived than virus-reactive T cells[48,49]. UB-612 induces a Th1-predominant IFN-g^+-T cell response (**Supplementary Figs. 5, 6**), which confirmed that the precision-designed Th/CTL peptide pool is essential and principally responsible for the T cell responses (**Fig. 7**) especially when a more focused "S-1 RBD" functional domain, lacking major Th/CTL epitopes, is used as its B immunogen component. Furthermore, induction of IFN-g-secreting SARS-CoV-2-specific T cells is present in patients with mild disease (as opposed to the severe disease) and accelerated viral clearance[50]. These observations are in line with the unique design of the UB-612 vaccine to combine S1-RBD-sFc and T-cell epitope peptides (the Th/CTL peptide pool) as core immunogens for a balanced activation of memory B-cell and T-cell immunities.

Overall, UB-612 vaccination (100 mg dose group) can induce substantial viral neutralizing antibodies with a long half-life (**Fig. 4b**) that go parallel with a long-lasting cellular immunity (**Fig. 7**). As memory B and T cells are critical in secondary responses to infection, the success of vaccines must depend on the generation and maintenance of immunological memory[14,15], and on rapid recall of effective humoral and cellular responses upon natural exposure or vaccine boosting.

Safety is an important concern, especially for vaccines that will be given to billions of people and may require intermittent or even annual vaccinations. Adenoviral vectors and mRNA are innovative technologies that have only been used widely in the context of the COVID-19 pandemic. These vaccines are associated with local and systemic reactogenicity, that is sometimes more severe after repeat dosing and they have been associated with rare but serious adverse reactions including myocarditis, Guillain-Barre syndrome, and thrombosis-thrombocytopenia[51]. While UB-612 has not yet been deployed widely enough to reveal rare adverse events, its composition (protein and peptides, with only aluminum adjuvant) suggests that it may have a good safety profile. So far, with >3900 subjects vaccinated, UB-612 has been shown to be very well tolerated with acceptable reactogenicity on repeated dosing.

While UB-612 has demonstrated induction of a striking neutralizing immunity against Delta variant, we understand the limitation of the small sample size of participants and the lack of booster data for the elderly and high-risk groups who have decreased immunity[52]. An additional booster vaccination study in our extended study of phase-2 trial [ClinicalTrials.gov: NCT04773067] are ongoing with about 3,500 participants aged 18-85 years to fully demonstrate the unique feature of UB-612 and its benefit to offer potent B and T immunity against multiple Variants of Concern (VoCs) including Delta in the coming months.

Methods

Trial design and oversight. Safety and immunogenicity of UB-612 vaccine were evaluated in an open-label phase-1 study, conducted at China Medical University Hospital,
Taiwan [ClinicalTrials.gov: NCT04545749] and an 84-day extension study to evaluate a 3rd booster dose [ClinicalTrials.gov: NCT04967742] (**Fig. 1; Table 1**). The primary-series 196-day Phase-1 study enrolled 60 healthy adults aged 20-55 years, who received two intramuscular (IM) injections (28 days apart) of 10-, 30- or 100-μg (N = 20/group). Seven to nine months following completion of the primary series, 50 participants were enrolled in the extension study to receive a booster-dose of 100 mg UB-612, with an interim analysis at 14 days and were also monitoring until 84 days post-booster.

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 authors 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 trial design, inclusion and exclusion criteria, conduct, oversight, and statistical analyses are available in the study protocols.

Trial procedures and safety. The phase-1 trial was initiated with a sentinel group of 6 participants to receive the low 10-μg dose, followed with the remaining 14 participants if without vaccine-related ≥ grade 3 adverse reaction. The same procedure was extended for the escalating 30- and 100-μg dose groups. Additional follow-up visits were scheduled for all participants on Days 14, 28, 35, 42, 56, 112, and 196. Study participants were scheduled for visits 14 and 84 days after the booster. Electronic diaries were provided to the participants to be completed for the 7-day period after each injection to record solicited local reactions at the injection site (pain, induration/swelling, rash/redness, itch, and cellulitis) and solicited systemic reactions (17 varied constitutional symptoms). Severity was graded using a 5-level (0 to 4) scale from none to life-threatening. In addition, participants recorded their axillary temperature every evening starting on the day of the vaccination and for the 6 subsequent days. Complete details for solicited reactions are provided in the study protocols [Supplementary Appendix].

Vaccine product. UB-612 is a multitope vaccine to activate both humoral and cellular responses (**Supplementary Fig. 1**). 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. This mixture of Th/CTL peptides is designed to elicit T cell activation, memory recall, and effector functions similar to that of natural infection with SARS-CoV-2. The S1-RBD-sFc fusion protein incorporates both linear and conformational epitopes and induces neutralizing antibodies. The two immunogen components are formulated with CpG1, a proprietary oligonucleotide (ODN). The vaccine product contained small amount of CpG1 at ≦2 mg, which is not likely high enough to serve as an immunostimulatory agents. Instead the CpG acts as an excipient to bind the peptide components through dipolar charge interactions into an immunostimulatory complex. The protein-pepide complex is then adsorbed to aluminum phosphate (Adju-Phos®) adjuvant, which promotes the activation of antigen-presenting cell pathways to induce an optimal immunogenicity profile intended to prevent infection with SARS-CoV-2. The UB-612 vaccine product is stored at 2 to 8 ^{o}C.

Immunogenicity. The primary immunogenicity endpoints were the geometric mean titers (GMT) of neutralizing antibodies against SARS-CoV-2 wild-type (Wuhan strain), and post-booster effects against Delta variant were explored as well. Viral-neutralizing antibody titers that neutralize 50% (VNT50) of live SARS-CoV-2 wild-type (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).

The secondary immunogenicity endpoints include binding IgG antibody responses to S1-RBD, inhibitory titers against S1-RBD:ACE2 interaction, and T-cell responses assayed by ELISpot and Intracellular Cytokine Staining (ICS). 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). The RBD IgG ELISA was fully validated using internal reference controls and results expressed in end-point titers. A panel of 20 human convalescent serum samples from COVID-19 Taiwan hospitalized patients aged 20 to 55 years were also tested for comparison with those in the vaccinees. 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. 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 SARS-CoV-2-USA WA 1/2020) and Delta variant (SARS-CoV-2-Taiwan-CDC#1144, B.1.617.2), which was conducted in P3 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 1.5×10^4 cells/100 µL/well. Plates are incubated at 37^{o} 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 TCID50) 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 VNT50 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. In seroconversion detected by live virus neutralization test, it was defined as a 4-fold change in antibody titer from baseline.

In a separate study with D614G, Alpha B.1.1.7, Gamma P.1, Beta B.1.351, and Delta B.1.617.2, the CPE assay was conducted at Viral and Rickettsial Disease Laboratory, State of California Department of Public Health USA. Neutralizing antibody titers were measured by CPE-based live virus neutralization assay using cells challenged with SARS-CoV-2 variants. The study was conducted in BSL3 lab at Viral and Rickettsial Disease Laboratory (VRDL), California State Department of Public Health (CDPH), USA. SARS-CoV-2 virus specific neutralization against SARS-CoV-2 variants titers were measured by *in vitro* microneutralization assay using Vero-81cells challenged with SARS-CoV-2 variants. Vero-81 were cultured with MEM supplemented with 1x penicillin-streptomycin (Gibco) and glutamine (Gibco) and 5% Fetal calf serum (Hyclone). Determination of SARS-CoV-2 virus specific neutralization titer was to measure the neutralizing antibody titer against the viruses based on the principle of VNT50 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. In seroconversion detected by live virus neutralization test, it was defined as a 4-fold change in antibody titer from baseline.

Inhibition of S1-RBD binding to ACE2 by ELISA. The 96-well ELISA plates are coated with 2 µg/mL ACE2-ECD-Fc antigen (100 mL/well in coating buffer, 0.1M sodium carbonate, pH 9.6) and incubated overnight (16 to 18 hr) at 4 °C. Plates are 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 are blocked by 200 µL/well of blocking solution (5 N 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) are mixed with 1:100 dilution of S1-RBD-HRP conjugate (horseradish peroxidase-conjugated recombinant protein S1-RBD-His), incubated for 30±2 min at 25±2 °C, washed and TMB substrate (3,3',5,5'-tetramethylbenzidine 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 450nm 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 ran below 0.16 μg/mL was defined as half of detection limit. Samples with titer exceed 2.5 μg/mL were further diluted for reanalysis.

Anti-S1-RBD binding IgG antibody by ELISA. The 96-well ELISA plates were coated with 2 μg/mL recombinant S1-RBD-His protein 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. 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 are washed six times with 200 μL Wash Buffer (PBS-0.05% Tween 20, pH 7.4). Bound antibodies were detected with HRP-rProtein A/G at 37 ^{O}C for 30 min, followed by six washes. Finally, 100 μL/well of TMB (3,3',5,5'-tetramethylbenzidine) prepared in Substrate Working Solution (citrate buffer containing hydrogen peroxide) was added and incubated at 37 ^{O}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, SpectraMax M2e). 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 0.5).

T cell responses by ELISPOT. Human peripheral blood mononuclear cells (PBMCs) were used in the detection of T cell response. For the primary-series 196-day Phase 1 study, antigen-specific interferon-gamma (IFN-γ) measurement to assess cellular (T cell) immune response were measured by ELISpot method by using human IFN-γ ELISpotPLUS kit (ALP) (MABTECH). For the booster-series third-dose series extension study, ELISpot assays were performed using the human IFN-γ/IL-4 FluoroSpot^{PLUS} kit (MABTECH). Aliquots of 250,000 PBMCs were plated into each well and stimulated, respectively, with pools of S1-RBD+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 unit (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 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 response. Upon completion of staining, cells were analyzed in a FACSCanto II flow cytometry (BD Biosciences) using BD FACSDiva software.

Statistics. As the studies were not powered for formal statistical comparisons of between-dose and between-phase vaccination, we report descriptive results of safety and immunogenicity. Immunogenicity results for GMT in titer are presented with the associated 95% confidence intervals. Seroconversion is defined as ≥ 4-fold increase in antibody titers from baseline. 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. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Declarations

Author contributions:

CYW, KPH, DYHS, and JHH conceived and designed the trial. DYHS, KPH, TK, and DGH contributed to the protocol and design of the study. KPH was the study site principal investigator. HKK, KLH, CYC, MSW, YTY, MHJ, HYS, YRL, PYC, YLL, JJL, CCL, YCC, MKM, and CVH were responsible for laboratory testing and assay development. KPH, DYHS, WJP, HKK, HTW, HL, JHH, and HCC contributed to the implementation of the study. SCTC onducted the statistical analysis. SD, ZL, WJP, and HTW were responsible for vaccine manufacturing and quality control. CYW, TPM, FG, MH, TK, DGH, HKK, and WJP were responsible for vaccine development. CYW, HKK, BSK, C-CK and TPM contributed to the preparation of the report. All other authors contributed to the implementation of the study and data collection. All authors critically reviewed and approved the final version.

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. WJP, FL, SD are named as co-inventors on a patent application covering this SARS-CoV-2 vaccine. MMH is the founder and shareholder of Vaxxinity which holds marketing rights for UB-612. HJY, DGH, TK, FG, MH, and TPM are employees of Vaxxinity and hold share options in Vaxxinity. DGH, TK and TPM are consultants to Public Health Vaccines LLC which has grants outside of the submitted work. TPM reports consulting fees from Merck and Memgen outside of the submitted work. TPM reports holding shares in Vaxess, Inc. which is engaged in development of a SARS-CoV-2 vaccine delivery device.

The study protocol is provided in the appendix. Individual participant data will be made available when the trial is complete, upon requests directed to the corresponding author; after approval of a proposal, data can be shared through a secure online platform.

Acknowledgements:

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China Medical University Children’s Hospital, and StatPlus Taiwan Corp. Special thanks are also extended to the clinical associates and the CMC task forces from both United BioPharma, Inc. and UBI Pharma, Inc, Members of the safety monitoring committee;Team members at Institute of Biomedical Sciences, Academia Sinica for the live virus neutralization assay; and Team members at the RNAi Core Facility, Academia Sinica for the pseudovirus neutralization assay. All health convalescent sera were supplied by Biobank at the National Health Research Institutes (NHRI), Taiwan. Finally, special administrative support by Jalon Tai, LK Huang and Fran Volz from the UBI group are also acknowledged with gratitude.

References

References are in the supplementary files section.

Tables

Tables 1-2 are in the supplementary files section

Figures

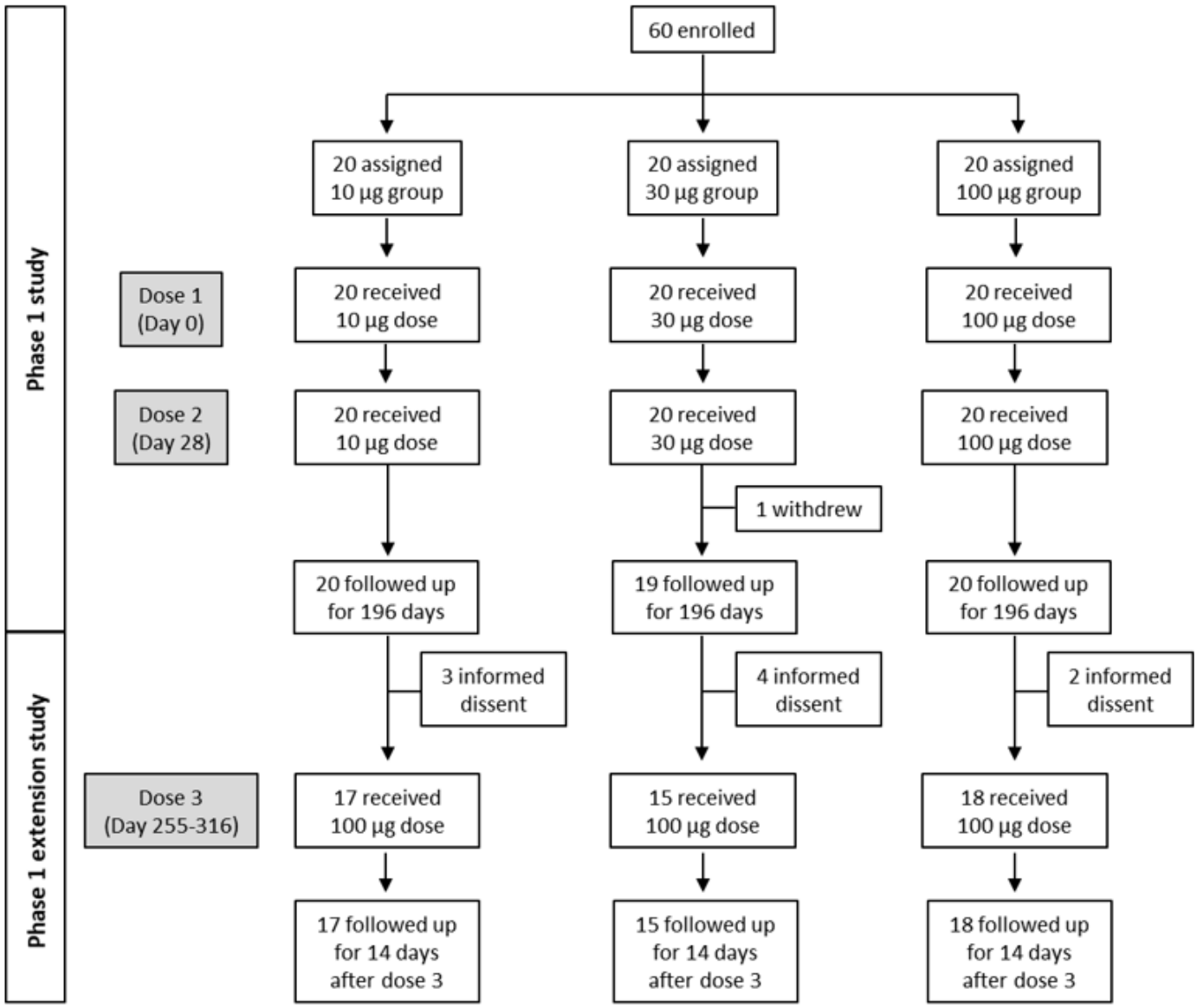


Figure 1

Flow of the primary 2-dose series in the phase-1 trial of UB-612 trial with extended booster third-dose study. Sixty healthy young adults, male and female, aged 20 to 55 years were enrolled for the primary series of the open-label, 196-day phase-1 study of UB-612 [NCT04545749], conducted between September 21, 2020 and May 24, 2021. They were administered intramuscularly with two vaccine doses at 10, 30, or 100 µg. All, but one, participants completed the study. The extension study [NCT04967742] that involved 50 enrolees was conducted between Days 255 to 316, a time period over 6 months after the second vaccine shot. The 50 participants in 10-µg (n = 17), 30-µg (n = 15), and 100-µg (n = 18) dose groups received a booster UB-612 dose at 100 µg and were followed up for 14 days for interim evaluation. They are to be monitored until 84 days post-booster.

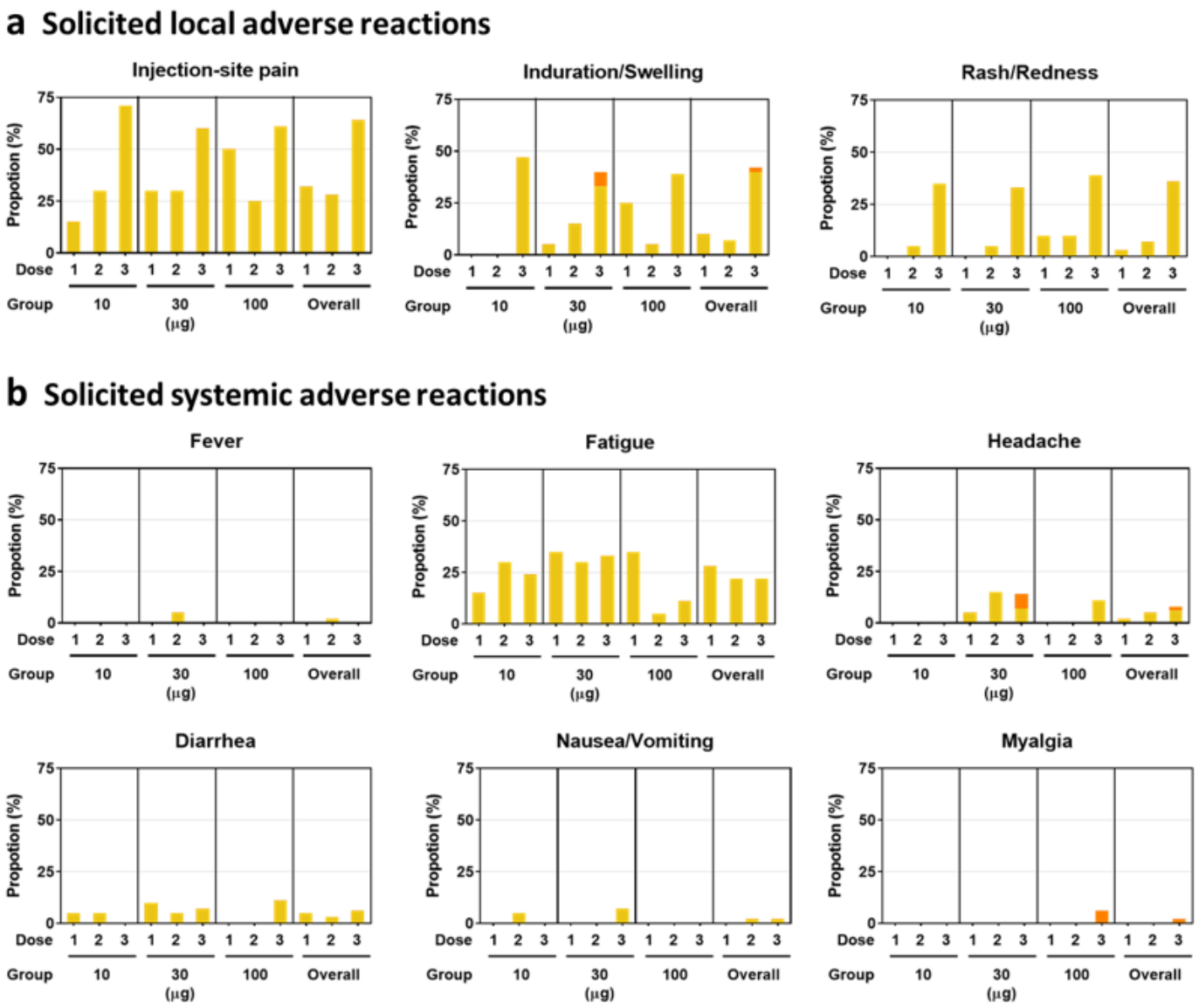


Figure 2

Selected solicited local and systemic reactions within 7 days of each vaccination three different doses of UB-612 vaccine. Both local and systemic reactions are shown as the percentage of participants who reported grade 1 (mild; yellow) or grade 2 (moderate, orange) for (a) local and (b) systemic adverse reactions. For dose 1 and dose 2, there were 20 participants in each dose group receiving 2 doses of UB-612 at 10, 30, or 100 μg. For the booster dose 3 at 100 μg, there were 17, 15, and 18 participants who originally assigned to 10-, 30-, and 100-μg dose group, respectively.

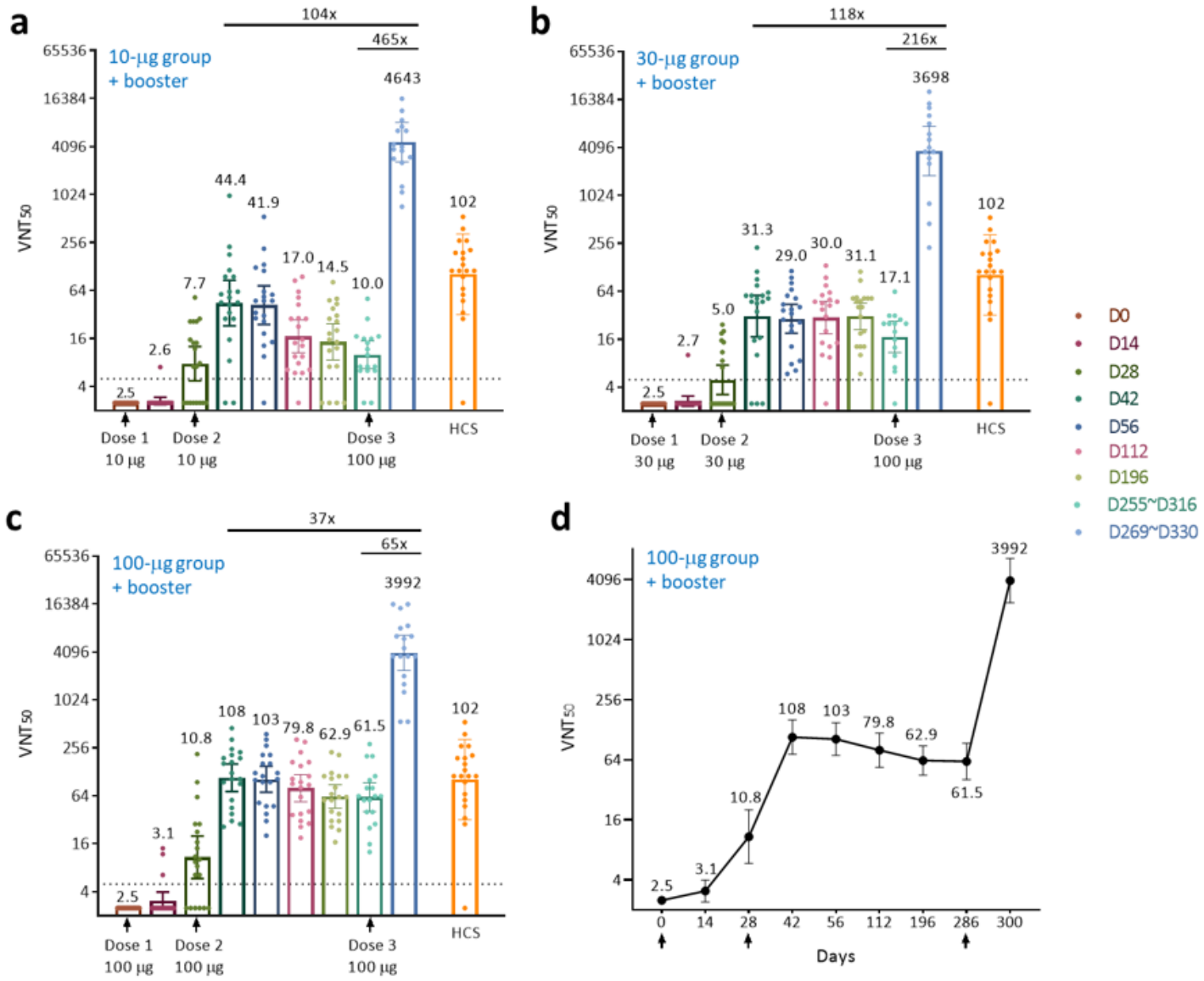
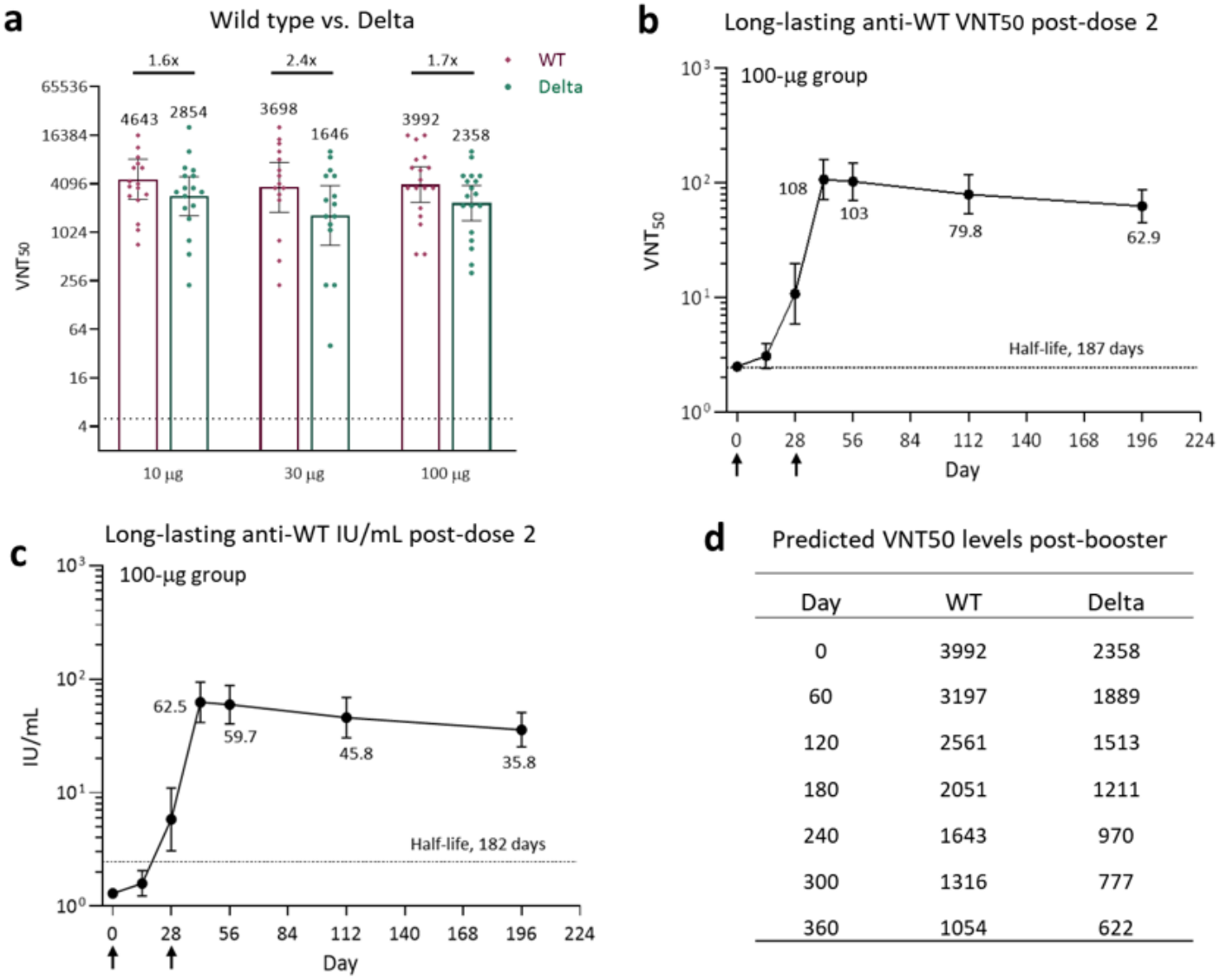


Figure 3

Viral-neutralizing antibody titers expressed in VNT50 are shown against live SARS-CoV-2 wild type after the primary 2-dose vaccination and the booster third-dose. In the primary 2-dose vaccination series of the 196-day phase-1 UB-612 trial, 60 participants were enrolled for the10-μg, 30-μg, and 100-μg dose groups (n = 20 each group), of which 50 participants were enrolled for the extension study where participants received a booster 3rd-dose at 100 μg (n = 17 for the 10-μg; n = 15 for the 30-μg, and n = 18 for the 100-μg dose group). The viral-neutralizing antibody geometric mean titers (GMT, 95% CI) that inhibit 50% of live SARS-CoV-2 wild-type (WT, Wuhan strain) were measured and expressed as VNT50 for (a) the 10-μg, (b) 30-μg, and (c) 100-μg dose groups. (d) Illustrated with the 100-μg dose group, the VNT50 data were recorded on Day 0 (pre-dose 1), Day 14 (14 days post-dose 1), Day 28 (1 mon. post-dose 1; pre-dose 2), Day 42 (14 days post-dose 2), Day 56 (1 mon. post-dose 2), Day 112 (3 mon. post-dose 2), Day 196 (6 mon. post-dose 2), Days 255 to 316 (pre-dose 3, the pre-booster), and Days 269 to 330 (14 days post-booster) for study participants of the three dose groups. The International Unit (IU/mL) corresponding to

50% neutralizing GMT and 95% CI (VNT50) is shown in the Supplementary Fig. 2. The titers for individual participants are shown by the circles. The horizontal dotted lines indicate the lower limit of quantification (LLOQ). HCS: human convalescent serum samples in the control group (n = 20).



d Predicted VNT50 levels post-booster

Day	WT	Delta
0	3992	2358
60	3197	1889
120	2561	1513
180	2051	1211
240	1643	970
300	1316	777
360	1054	622

Figure 4

UB-612 produced potent, long-lasting viral-neutralizing titers against SARS-CoV2 wild-type and Delta variant and predicted post-booster VNT50 levels over 12 months. In the primary 2-dose series (Days 0 and 28) of the 196-day phase-1 trial and the extended booster 3-rd dose administered at mean Day 286 (Days 255-316), (a) the time course of 50% viral-neutralizing geometric mean GMT titers (VNT50, 95% CI) were recorded for study participants (n = 18) in the 100-µg dose group, to whom the booster dose at 100 µg stimulated a profound VNT50 level, reaching at 3992 against live SARS-CoV-2 wild type (WT), (b) and reaching at 2358 against live Delta variant 14 days post-booster. Similar high anti-WT and ant-Delta VNT50 levels were observed for the lower 30- and 10-µg dose groups. (c) The anti-WT neutralizing VNT50 titers decayed slowly with a half-life of 187 days, based on the first-order exponential model fitting

(SigmaPlot) over Days 42-196 (R2 = 0.9881; the decay rate constant Kel = -0.0037; half-life = 0.693/Kel). (d) Assuming the half-lives estimated in the primary series are applicable for the post-booster phase, and calculated by decay functions (VNT50)Day = (VNT50)0 x [e(-0.0037xDay)] for WT and Delta, the titers 3992 of WT and 2358 of Delta on Day 0 are predicted to decline to the levels of 1054 and 622 on Day 360, respectively. With further exponential decay till Day 721, the respective VNT50 levels would remain at 278 for WT and 164 for Delta.

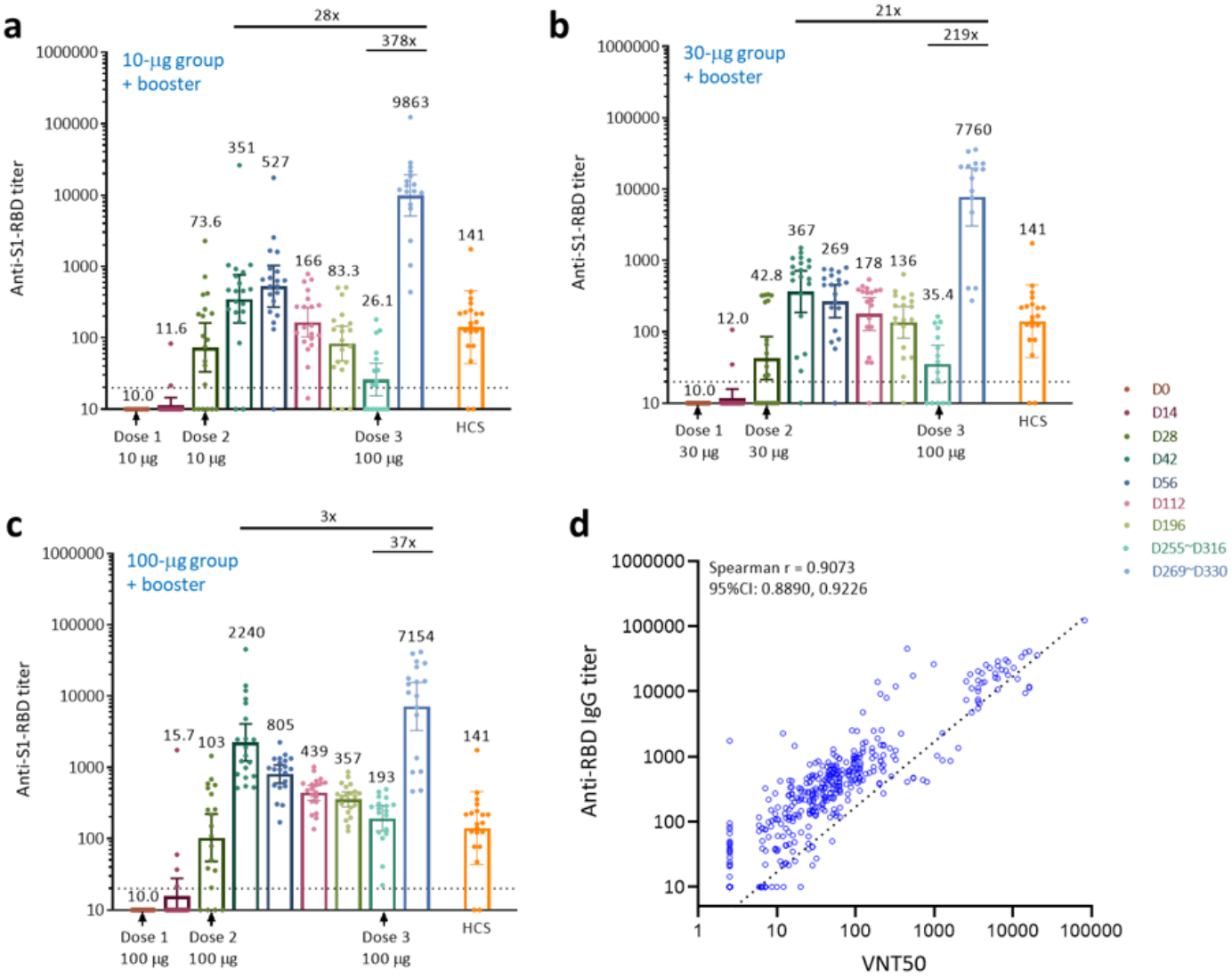


Figure 5

Anti-S1-RBD IgG binding titers on ELISA in the primary 2-dose vaccination and after the booster third-dose. ELISA-based anti-S1-RBD antibody binding titers in the primary 2-dose vaccination series of a 196-day phase 1 trial (60 participants) and in the extension study with a booster third-dose. Participants of (a) 10-μg, (b) 30-μg, and (c) 100-μg dose groups (n = 20 each dose group) who had received two assigned vaccine doses, 28 days apart, and a booster third dose of 100 μg at a time over 6 months administered to 50 participants (n = 17 for the 10-μg, n = 15 for the 30-μg, and n = 18 for the 100-μg dose groups). Serum samples were collected at the indicted time points for measuring anti-S1-RBD antibody binding by ELISA,

expressed as geometric mean titer GMT and 95% CI. The horizontal dotted lines indicate the lower limit of quantification (LLOQ). (d) Good correlation exists between anti-S1-RBD antibody binding and VNT50. Data are plotted for all prime/boost vaccinated participants (10-, 30- and 100-µg dose groups). Data points for participants at day 0 are excluded from correlation analysis. Correlation analyzed by Nonparametric Spearman correlation method.

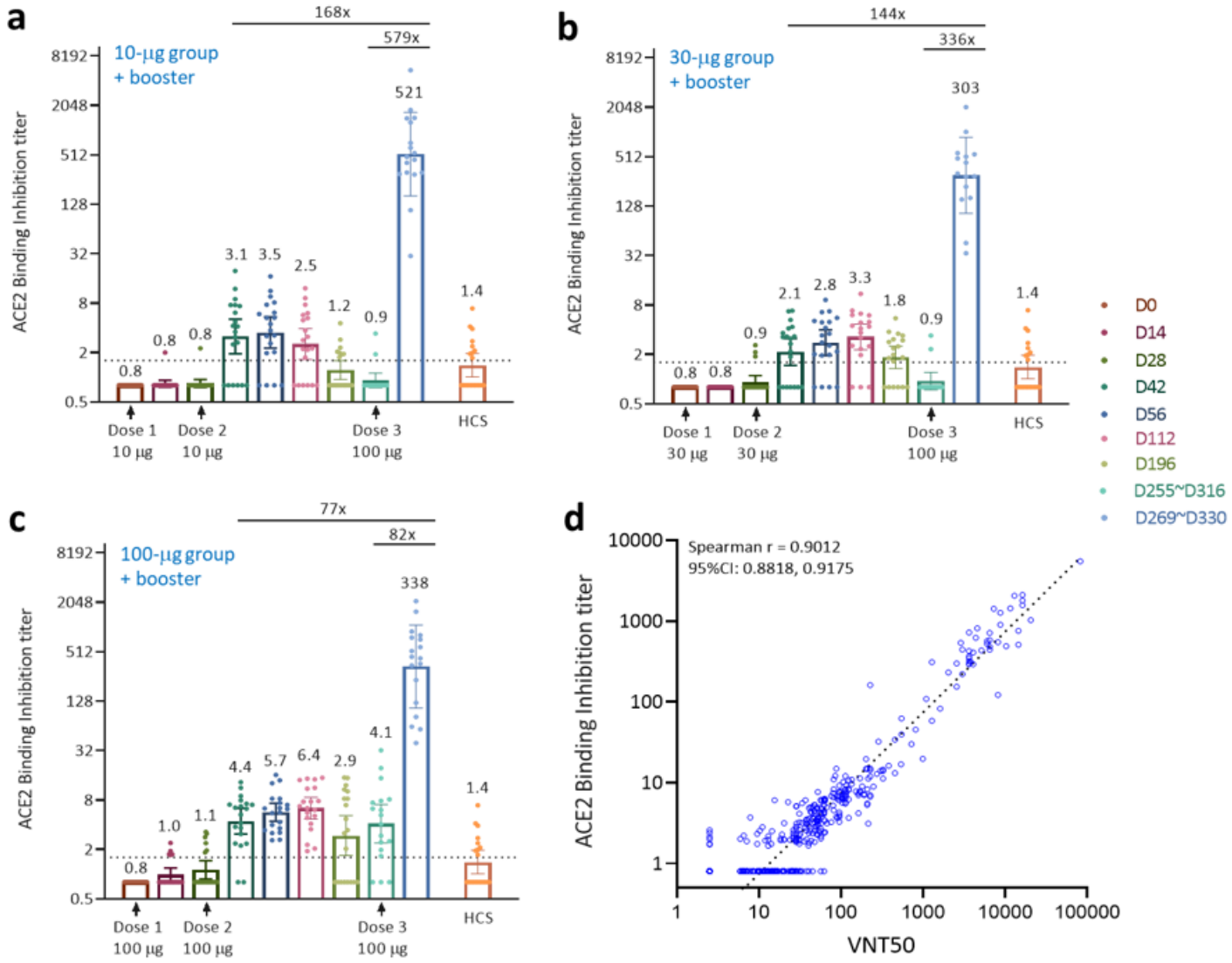


Figure 6

Inhibition titers against S1-RBD:ACE2 binding on ELISA in the primary 2-dose vaccination and after the booster third-dose. ELISA-based neutralization (inhibition) of S1-RBD:ACE2 binding titers in the primary 2-dose vaccination series of a 196-day phase 1 trial (60 participants) and in the extension study with a booster third-dose. Participants of (a) 10-µg, (b) 30-µg, and (c) 100-µg dose groups (n = 20 each dose group) received two assigned vaccine doses, 28 days apart, and a booster third dose of 100 µg at a time over 6 months administered to 50 participants (n = 17 for the 10-µg, n = 15 for the 30-µg, and n = 18 for the 100-µg dose groups). Serum samples were collected at the indicted time points for measuring the inhibition titers against S1-RBD binding to ACE2 by ELISA. The horizontal dotted lines indicate the lower

limit of quantification (LLOQ). (d) Good correlation exists between S1-RBD:ACE2 binding inhibition and VNT50. Data are plotted for all prime/boost vaccinated participants (10-, 30- and 100-µg dose groups). Data points for participants at day 0 are excluded from correlation analysis. Correlation analyzed by Nonparametric Spearman correlation method.

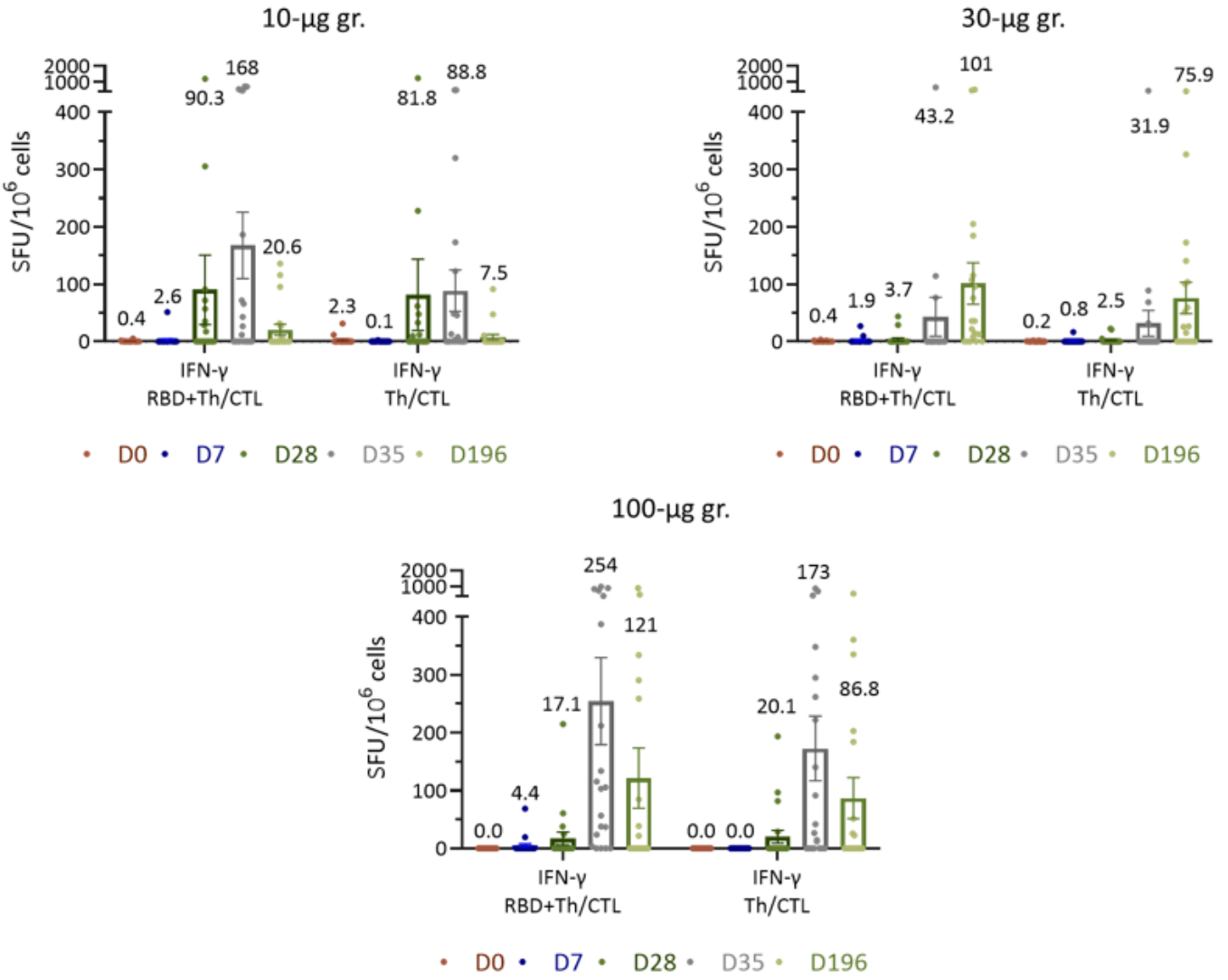


Figure 7

T-cell response by IFN-γ ELISpot after restimulation with RBD+Th/CTL or Th/CTL in the primary 2-dose vaccination series of the 196-day Phase-1 trial. T-Cell responses were measured by IFN-γ ELISpot with PBMC cells from young adults (20 to 55 years) in the phase-1 trial of UB-612 with three dose groups of 10, 30, and 100 µg (n = 20 each). The Y-axis shows the number of cytokine secreting cells per 106 PBMC cells in ELISpot assays and the X-axis depicts IFN-γ (Th1) response to restimulation with S1-RBD+Th/CTL and IFN-γ response to pooled Th/CTL peptides. Brown dots represent Day 0, blue dots represent Day 7, dark green dots represent Day 28 (before second vaccination), gray dots represent Day 35 and green dots Day 196. Of note, in both stimulation cases, the T cell responses (SFU/1.0x106 cells) at Day 196 retained half (50%) that at Day 35, i.e., 121-vs.-254 for RBD+Th/CTL and 86.8-vs.-173 for

Th/CTL. This suggests the immune T-cell responses in vaccine recipients are substantially long-lasting, over 6 months. In addition, the bulk of the T cell responses (~70%) were contributed by the presence of Th/CTL as compared with RBD+Th/CTL.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- References.docx
- UB612boostermanuscriptSupplementaryAppendix.docx
- Tables.docx