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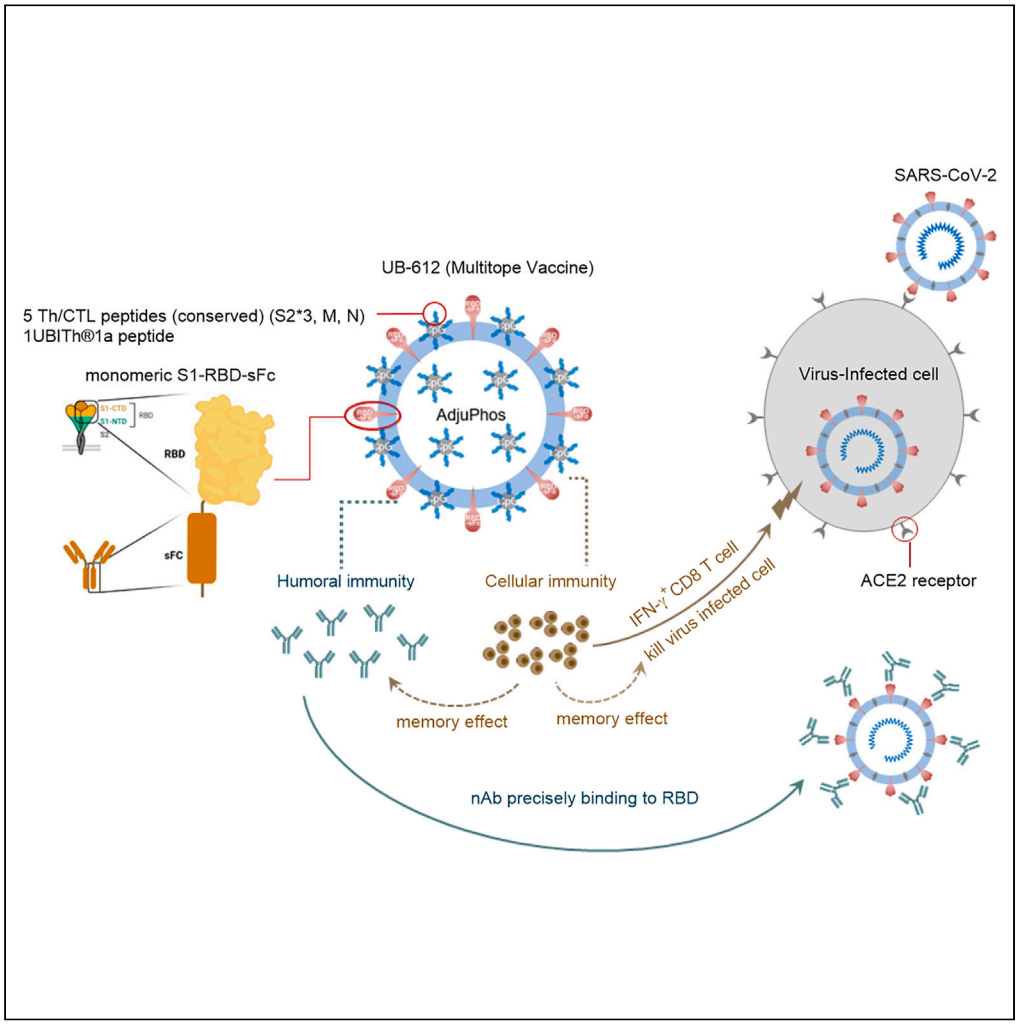

Chang Yi Wang, Be-Sheng Kuo, Yu-Hsiang Lee, ..., Hsi-Chi Chang, Lin-Fang Fu, Wen-Jiun Peng

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Highlights

Targets RBD and multiple conserved T cell epitopes on Spike and non-Spike proteins

0 cases of hospitalization and ICU ≥14 months post-2nd dose, or ≥10 months post-booster

Potent memory cytotoxic $CD8^+$ T cell immunity pivots the control of disease severity

UB-612 may be a competent primer and booster for persons vulnerable to infection

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UB-612 pan-SARS-CoV-2 T cell immunity-promoting vaccine protects against COVID-19 moderate-severe disease

Chang Yi Wang,[1,2,*] Be-Sheng Kuo,[1] Yu-Hsiang Lee,[1] Yu-Hsin Ho,[1] Yi-Hua Pan,[1] Ya-Ting Yang,[1] Hsi-Chi Chang,[1] Lin-Fang Fu,[1] and Wen-Jiun Peng[1]

SUMMARY

UB-612 pan-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine targets the monomeric Spike S1-receptor binding domain (RBD) subunit protein along with five sequence-conserved T cell epitopes found on Spike S2 and non-Spike M and N proteins. UB-612 vaccination safely induces potent, broad, and long-lasting immunity against SARS-CoV-2. A phase-2 trial-extended observational study during the Omicron BA.2-/BA.5-dominated outbreak was conducted to investigate UB-612's protective effect against COVID-19 hospitalization and intensive care unit (ICU) admission (H-ICU). Additionally, memory viral-neutralizing titer and T cell immunity behind disease protection were explored. No cases of H-ICU were reported beyond 14 months post-second dose or beyond 10 months post-booster (third dose). The positive outcome correlates with strong cytotoxic CD8 T cell immunity, in line with the results of an ongoing phase-3 heterologous booster trial showing that UB-612 can enhance anti-BA.5 seroconversion rate and viral-neutralizing titer for mRNA, adeno-vectored, and virus-inactivated vaccine platforms. The UB-612 multitope vaccine may serve as an effective primer and booster for those at risk of SARS-CoV-2 infection.

INTRODUCTION

The current decline in COVID-19 cases indicates a positive shift in the trajectory of the pandemic, marking a turning point after a three-year-long catastrophe. This was underscored by the World Health Organization's declaration on May 5, 2023,[1] officially ending the global "health emergency," as both mild and severe infection numbers dropped to their lowest levels. However, it is important to note that the pandemic may not be fully behind us,[2–6] as Omicron XBB-related variants, which can evade antibodies, continue to evolve and circulate even in countries with high vaccination rates. Additionally, the once-prevalent XBB.1.5 variant has been surpassed by the EG.5 variant, which is currently the dominant strain in the USA and worldwide (Figure S1). Concurrently, the increasing presence of the EG.5 variant (Figure S2), as detected in wastewater, is a significant contributing factor to the recent uptick in hospitalizations and fatalities.[7]

The recently identified BA.2.86 variant, characterized by the highest number of mutations observed in a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant to date, contains 34 and 30 mutation sites in comparison to BA.2 and XBB.1.5, respectively (Table S1). However, global cases of BA.2.86 infection remain rare; it exhibits even lower immune evasion capability than initially feared.[8] In fact, the neutralizing antibody titer against BA.2.86 showed comparable or slightly higher levels relative to XBB.1.5 and EG.5; and the variant with the lowest neutralizing titer is FL.1.5.1,[9–12] which is considerably less mutated than BA.2.86 (Table S1). This indicates that the sheer number of mutation sites alone does not necessarily determine the strength of antibody evasion. As of now, there is no evidence suggesting significant differences in symptom scope and disease severity between the various Omicron subvariants.[7,13,14]

Relative to the Delta strain, members of the Omicron family inherently pose a weaker threat to lung tissues and thus a lower pathogenicity. This is likely due to inefficient cell-to-cell syncytia formation upon contact with the upper respiratory membrane, and a distinct cell entry mechanism that does not require the fusogenic co-receptor TMPRSS2.[15–17] However, Omicron-related COVID-19 is not bound by seasonal patterns[18] and is certainly more lethal than the seasonal flu.[19,20] Additionally, with the emergence of increasingly contagious Omicron variants, there is a potential for them to overcome immunity conferred by vaccination, prior infection, or hybrid immunity, posing a risk even to highly vaccinated individuals.[21] Furthermore, any reinfection, regardless of severity, would heighten the risks of mortality, hospitalization, and other health complications, including burden of long COVID.[22,23] This means that there is no risk-free scenario for COVID-19 reinfection, especially for the elderly, immunocompromised individuals, and those with underlying health conditions.

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While no longer deemed a public health emergency, the World Health Organization emphasized that COVID-19 remains a global health threat. This could arise from the emergence of "another variant," potentially leading to new waves of disease and fatalities, or the emergence of "another pathogen" with even greater deadly potential.[24] These possibilities suggest two scenarios: first, through natural evolution,[25] a more pathogenic virus could arise, surpassing the Omicron lineage; and second, through gain-of-function genetic manipulation, the catastrophic viral variants could be created in a laboratory setting.[26–30] Both scenarios could exacerbate systemic infection and trigger unforeseen outbreaks of COVID. To prevent the next potential pandemic catastrophe, it is imperative to develop versatile pan-SARS-CoV-2 vaccines that target multiple sequence-conserved epitopes across both Spike and non-Spike proteins of SRAS-CoV-2.

Research confirms that viral Spike protein governs infectivity, whereas non-Spike proteins drive virulence, determining the severity of disease and mortality.[27,31] Immune responses generated from vaccines targeting T cell epitopes on non-Spike proteins play a crucial role in triggering an immediate interferon (IFN) response and establishing memory immunity, facilitating viral clearance through killer $CD8^+$ T lymphocytes.[32–37] This mechanism has the potential to prevent severe disease and reinfection altogether.

Most of the currently approved vaccine platforms have limitations in generating full-blown T cell immunity, as they primarily focus on the Spike protein alone as the sole immunogen. Targets on non-Spike proteins were grossly overlooked at the onset of COVID-19 vaccine development through the Operation Warp Speed [38] three years ago. For advance preparedness to avert and mitigate the potential impact of a new catastrophic outbreak, it is imperative to prioritize the development and validation of an innovative genre of next-generation vaccines, which stands as an urgent global objective. These should not merely be updated versions targeting Spike variants for frequent boosters, which could potentially disrupt or weaken the immune system.

UB-612 represents a new generation of pan-variant COVID-19 vaccine that has been demonstrated in both the phase-1 and a large-scale phase-2 trials to be safe and well tolerated without concerns of severe adverse events, producing potent, broad, and long-lasting B cell and T cell immunity.[39,40] In mice and macaque models, UB-612 can significantly reduce viral loads, lung pathology scores, and disease progression.[41]

In the present retrospective e-questionnaire observational clinical study conducted in Taiwan, an extension cohort from a clinical phase-2 trial, UB-612, exhibits a potential of providing pronounced, long-lasting (overall $\geq$12 months post-booster) protection against COVID-19 moderate-severe disease as shown by zero cases of hospitalization and ICU admission during the outbreak dominated by Omicrons BA.2 and BA.5.

UB-612 pan-SARS-CoV-2 vaccine is constructed with five designer peptides as immunogens to promote comprehensive T cell immunity (Table S2), which comprises sequence-conserved, non-mutable helper, and cytotoxic T lymphocyte (Th/CTL) epitopes derived from Spike (S2x3) and non-Spike Membrane (M) and Nucleocapsid (N) proteins across all SARS-CoV species, in addition to targeting the monomeric S1-RBD subunit protein (original ancestral strain) for induction of RBD-focused neutralizing antibody response. UB-612 vaccine is unique as it simultaneously targets epitopes on both Spike and non-Spike proteins.

RESULTS

Study participants for observation of vaccine effect against COVID-19

The UB-612 observational study (Figure 1) included participants aged 12–85 years, with an approximately equal distribution of genders (Table 1). They received at least the primary 2-dose series between March 23, 2021, and August 21, 2021, and were scheduled to receive a homologous booster (third dose) between October 16, 2021, and December 09, 2021 (Table S3).

Throughout the pre-Omicron years of 2020 and 2021, Taiwan effectively managed to contain the spread of COVID-19, reporting very low infection rates. The only exception was a brief surge in Alpha variant cases confirmed between April and July 2021. However, in late March 2022, a widespread outbreak of the highly contagious Omicron variants occurred (Figure 2), persisting through the end of the year. This outbreak was primarily driven by Omicron BA.2 and BA.5. This provided a valuable window of opportunity for assessment of protective effects of UB-612 vaccination against moderate-severe disease, specifically hospitalization and ICU admission, hereafter referred to as H-ICU.

As of September 30, 2022, it has been six months since the significant Omicron outbreak, which equates to at least 10 months after receiving the booster (third dose) and at least 14 months after the second dose (Table S3). Approximately 28% of Taiwan's population was confirmed to have been infected (Table S4), and this number increased to 36% by the end of the year (Figure 2B).

Out of the 2,900 enrollees who completed the questionnaire survey, only a limited number of participants adhered to the UB-612 vaccination regimen: 103 participants received the primary 2-dose series only, while 212 participants received the primary series along with 1 homologous booster dose (Figure 1; Table 1). This was primarily attributed to the official recommendation that the majority of participants should receive only Emergency Use Authorization (EUA)-approved heterologous booster over time.

Protection against moderate-severe disease (hospitalization and ICU admission)

As of September 30, 2022, among the 212 study participants who received 3 doses of UB-612 and were observed for at least 10 months post-booster, none reported any cases of H-ICU (0%) (Table 2). Additionally, all 103 participants who received only primary 2-dose series reported no cases of H-ICU cases (0%) as of $\geq$14 months after the dose.

As of September 30, 2022, the entire population of Taiwan who received EUA-authorized vaccines reported a dose-dependent decrease in H-ICU rates for moderate to severe disease (Figure 3A). These rates were 0.44%, 0.22%, 0.11%, and 0.06% for individuals unvaccinated, receiving 1, 2, or $\geq$3 EUA-vaccine doses, respectively.[42–44]

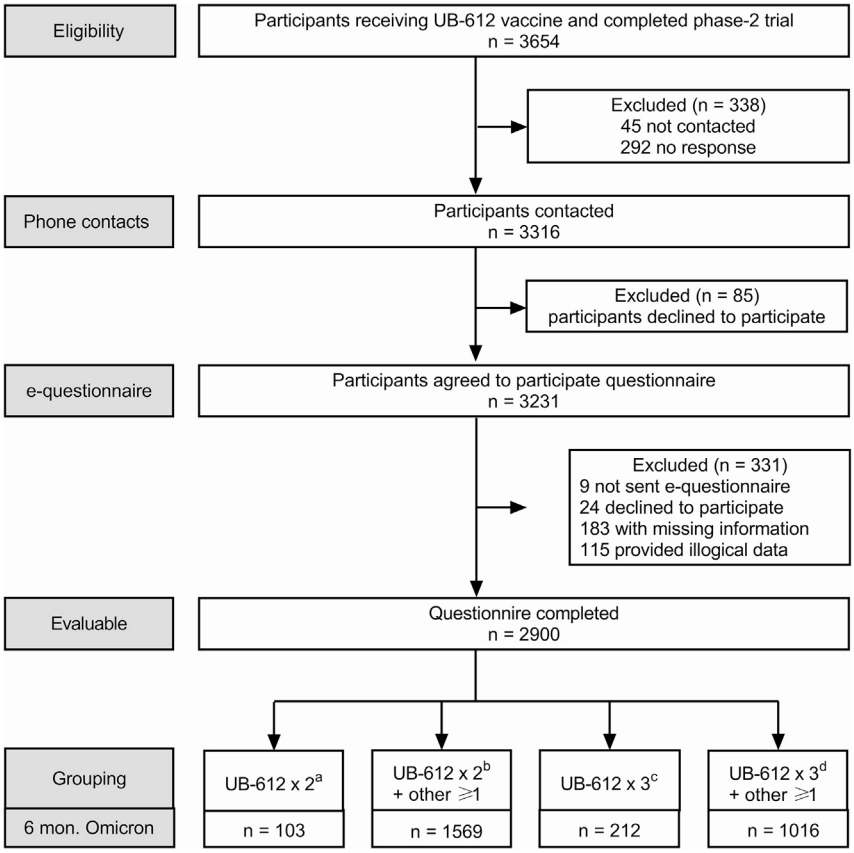

Figure 1. Flowchart of the retrospective questionnaire-based, observational study of UB-612 extended from the phase-2 trial primary and booster series

The web-based questionnaire observational study (V-205-Q) is a booster extension arm of the phase-2 trial (V-205) conducted in Taiwan from October 2022 to March 2023. Participants completing the study were grouped for evaluation of UB-612's protection effects against Omicron infection at September 30, 2022, the time 6 months after Omicron outbreak since late March 2022 (i.e., ≥10 months post-booster third dose) (Table 1). There were 4 groups: [a]who received only UB-612 primary 2 doses (n = 103); [b]UB-612 primary 2 doses plus more than 1 heterologous booster doses (n = 1,569); [c]UB-612 x 3, i.e., primary 2 doses plus 1 homologous booster dose (n = 212); and [d]three doses of UB-612 plus more than 1 heterologous booster doses (n = 1,016). The sample sizes for the participants who stayed clean on the UB-612 vaccination course were limited, n = 103 for the 2-dose and n = 212 for the 3-dose group, as volunteers were officially recommended by regulatory agency to receive only EUA-approved vaccines.

As of September 30, 2022, Taiwan reported an overall infection rate of approximately 30%, with a moderate-severe disease rate of 0.46% (Figure 2B). Notably, 65.4% of local population has received 3 doses or more of EUA-authorized vaccines (Figure 3B), primarily AZD1222, BNT162b2, and mRNA-1273 (Figure 3C). Unfortunately, specific details regarding vaccine regimen and vaccination status for this population pool were not available. The moderate-severe disease rate initially rose in tandem with the infection rate, peaking at 0.47% in July 2022, and subsequently leveling off through the end of the year (Figure 2B).

Infection rate after UB-612 vaccination

Following UB-612 vaccination, we initially observed a low infection rate of 1.2% (symptomatic and asymptomatic) as of May, 11, 2022 (6 months post-booster, which is approximately 6 weeks after the Omicron outbreak) (Table 3). The rate subsequently rose to 27.8% as of September 30, 2022 (≥10 months post-booster).

On the other end, the entire population of Taiwan, encompassing those who received EUA-vaccines and the unvaccinated, showed an infection rate of 2.2% (including both symptomatic and asymptomatic cases) observed on May 11, 2022 (approximately 6 weeks after the Omicron outbreak) (Table 3).[43,44] This rate increased to a similar approximate level of 27.9% as of September 30, 2022.

Vaccine immunity behind disease-protecting effect

In conjunction with the UB-612 observational study, we conducted an analysis of serum viral-neutralizing antibody titers recalled by the booster. This analysis encompassed highly contagious Omicron variants such as BA.5 and beyond (including XBB.1.5, BQ.1.1, and

Table 1. Demographics of participants in the questionnaire-based observational study for evaluation of protection effect by UB-612 vaccine

	All subjects (n = 2,900)	2 + 0[a] (n = 103)	3 + 0[a] (n = 212)	2 + X[a] (n = 1569)	3 + X[a] (n = 1,016)	p value[d]
Age[b]						<0.001
Median	40	31	44	37	43	
Range	12–82	12–77	20–80	12–82	19–82	
Age group[b]	<0.001					
12–17	299 (10.3%)	38 (36.9%)	0 (0.0%)	261 (16.6%)	0 (0.0%)	
18–64	2,059 (71.0%)	48 (46.6%)	159 (75.0%)	1,080 (68.8%)	772 (76.0%)	
≧65	542 (18.7%)	17 (16.5%)	53 (25.0%)	228 (14.5%)	244 (24.0%)	
Sex	<0.001					
Male	1,476 (50.9%)	68 (66.0%)	114 (53.8%)	754 (48.1%)	540 (53.1%)	
Female	1,424 (49.1%)	35 (34.0%)	98 (46.2%)	815 (51.9%)	476 (46.9%)	
BMI (kg/m^2)[b]	<0.001					
Median	24.2	22.7	25.2	24.0	24.8	
Range	13.1–50.9	15.9–44.7	15.8–42.9	13.1–50.9	16.7–50.1	
Obesity, n (%)[c]	0.7					
Obese	2,578 (88.9%)	12 (11.7%)	27 (12.7%)	190 (12.1%)	136 (13.4%)	
Non-obese	321 (11.1%)	91 (88.3%)	185 (87.3%)	1,378 (87.9%)	880 (86.6%)	
Comorbidity, n (%)	0.6					
Yes	62 (2.1%)	2 (1.9%)	4 (1.9%)	31 (2.0%)	25 (2.5%)	
No	2,838 (97.9%)	101 (98.1%)	208 (98.1%)	1,538 (98.0%)	991 (97.5%)	

The observation period was determined from the date of completing UB-612 primary series to 6 months after the Omicron outbreak in Taiwan, specifically until September 30, 2022, the date the study participants were categorized into five groups based on their vaccination status (Figure 1).

[a]2 + 0: individuals who had received only the UB-612 primary series; 3 + 0: individuals who had received the UB-612 primary series with a homologous booster; 2 + X: individuals who had received the UB-612 primary series with at least one heterologous vaccines; 3 + X: individuals who had received 3 doses of the UB-612 vaccine with at least one heterologous vaccines.

[b]Age and BMI were presented at the time of participating in the phase-2 clinical trial of the UB-612 vaccine.

[c]The BMI cutoff points for obesity vary by age. The BMI cutoff points for adolescents were provided by the Health Promotion Administration, Ministry of Health and Welfare. In adults, a BMI of 30 kg/m^2 or higher defines obesity.

[d]Kruskal-Wallis rank-sum test (age/BMI); Pearson's chi-squared test (sex/age group); Fisher's exact test (comorbidity).

CH.1.1), albeit with a limited availability of serum samples. We also adapted the previously profiled booster memory T cell responses[39] to investigate whether they could serve as a more accurate indicator of the vaccine's protective effect against disease severity.

Post-booster (third dose) humoral neutralizing antibodies against live viruses

In line with the phase-1 findings for post-booster live virus-neutralizing titers in participants aged 20–55 years (50% virus neutralisation titre (VNT_{50}) titers of 670/485 against Omicron BA.1/BA.2, respectively),[39] UB-612 demonstrated a similar, significant induction of neutralizing antibody against live virus titers in phase-2 booster study participants aged 18–85 years. These titers (VNT_{50}) were measured at 359/325 against BA.1/BA.2, and at 123 against BA.5 (Figure 4A). These values stand in stark contrast to those reported for other vaccine platforms, such as anti-BA.1 antibody titer falling within a lower range of 46.2–106 (Table S5). Notably, the corresponding VNT_{50} values for EUA-authorized vaccines against BA.2 and BA.5 have not yet been reported.

Since standardized neutralization methods have not been established for comparison, these data points (Table S5) are presented for contrast rather than for statistical comparison. Nevertheless, a clear trend of platform-dependent differences in live virus-neutralization potency against Omicron variants is discernible. This suggests a potential competitive advantage of UB-612 over other vaccine platforms, consistent with its previously observed potent neutralizing titers against the live Delta variant after booster vaccination, as reported in earlier studies.[40] These distinctive differences in neutralizing titer strength are also in alignment with the results obtained from pseudovirus assays contrasting UB-612 with other vaccine platforms.

Post-booster (third dose) humoral neutralizing antibodies against pseudoviruses

In contrast with other vaccine platforms, the phase-2 UB-612 booster vaccination demonstrated a notable advantage in terms of 50% pseudovirus-neutralizing titers ($pVNT_{50}$) against the original wild-type WT/BA.1/BA.2/BA.5 strains.[39] In our latest pseudovirus-neutralization assay, conducted against the new subvariants, we observed a sharp decline in $pVNT_{50}$ beyond the BA.5 variant, e.g., a 10-fold difference between

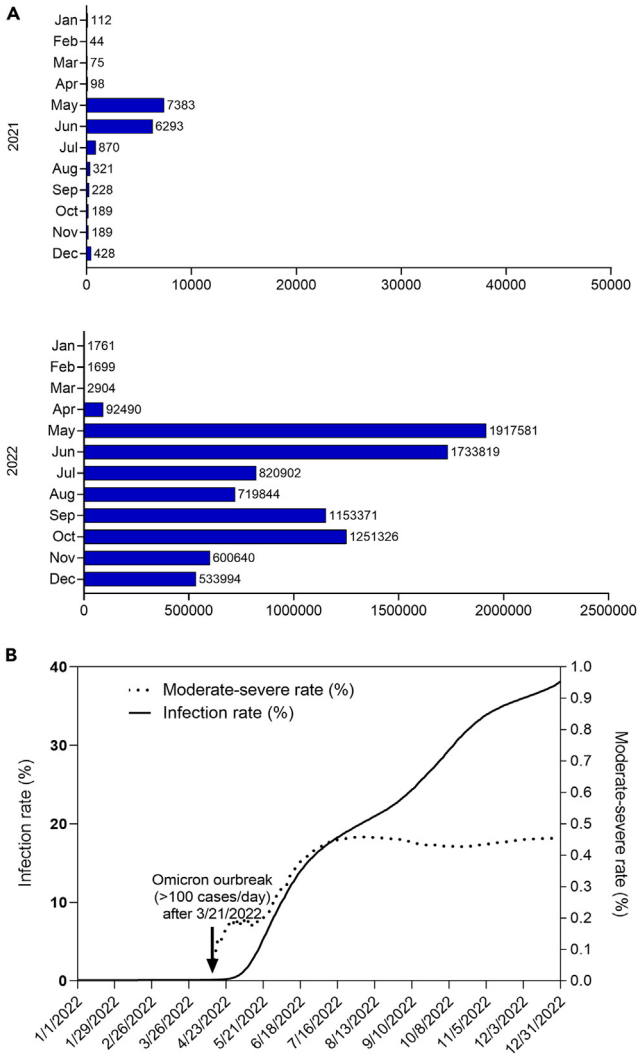

Figure 2. The 2022 outbreak of Omicrons and COVID-19 prevalence in Taiwan

(A) Very few COVID-19 cases were noted throughout the year 2021, except only a blip incidence of Alpha variant infection confirmed in April-July that year. The first COVID-19 outbreak occurred with Omicron variant in late March, 2022. The huge number of cases were recorded throughout the year end, mainly BA.2 (BA.2.75 and XBB not present) and BA.5 (BF.7 and BQ.1 not present). The daily confirmed COVID-19 cases in Taiwan were obtained from the website of Ministry of Health and Welfare (https://covid19.mohw.gov.tw/en/sp-timeline0-206.html).

(B) The COVID-19 cases rammed up staggeringly in April-May with the prevalence remained substantially high throughout the year end of 2022. The solid and dotted lines represent rates of infection (symptomatic and asymptomatic) and moderate-severe disease, respectively.

BA.5 and XBB.1.5. The induced $pVNT_{50}$ titers against BA.2/BA.5/XBB.1.5/BQ.1.1/CH.1.1 were measured at 1890/854/97/91/45, respectively (Figure 4B).

With regards to UB-612 vaccination, the results from live virus and pseudovirus-neutralization assays exhibit a robust correlation (Figure 4C). This is evident in the assays conducted against Omicron variants BA.1, BA.2, and BA.5, which show Spearman correlation coefficients of 0.866, 0.659, and 0.791, respectively. It is worth noting that no such correlation analysis between VNT_{50} and $pVNT_{50}$ has been presented for other vaccine platforms. Additionally, when considering protective humoral immunity, the measurement of the "live virus-neutralizing" titer (VNT_{50}) would carry more significance than the "pseudovirus-neutralizing" titer ($pVNT_{50}$). This is because the latter, an artificial assay, is based solely on the interaction with the viral outer Spike protein.

The mRNA vaccine platforms have shown varying booster-induced neutralizing titers ($pVNT_{50}$) against BA.2/BA.5/XBB.1.5/BQ.1.1/CH.1.1. For instance, a 3-dose monovalent mRNA vaccine reported titers of 475/197/59/25/8, while a 3-dose monovalent vaccine combined with a 1-dose bivalent mRNA vaccine showed titers of 2,151/1,099/241/86/66.[45] In all mRNA vaccine cases and UB-612 vaccine as well (Figure 4B), there is a consistent trend of a sharp decline in $pVNT_{50}$ from BA.5 to XBB.1.5. This pattern is further supported by other studies, which report a >5- to >10-fold reduction in pVNT50 for XBB.1.5 compared to BA.5.[9–12,46]

Table 2. COVID-19 disease severity observed ≥10 months post-booster (3rd-dose) and ≥14 months post-2nd dose of UB-612 vaccine in the observational study

Characteristics		Overall N = 2,900	SARS-CoV-2 infection status: Non-infection N = 2065	SARS-CoV-2 infection status: Infection N = 835	p value[a]	Severity: Asymptomatic N = 37	Severity: Mild N = 794	Severity: Moderate-severe N = 4	p value[b]
Sex					0.3				>0.9
Female		1,424 (49.1%)	1,002 (70.4%)	422 (29.6%)		20 (1.4%)	400 (28.1%)	2 (0.1%)	
Male		1,476 (50.9%)	1,063 (72.0%)	413 (28.0%)		17 (1.2%)	394 (26.7%)	2 (0.1%)	
Age Groups				<0.001					0.08
12–17		299 (10.3%)	209 (69.9%)	90 (30.1%)		2 (2.2%)	88 (97.8%)	0 (0.0%)	
18–64		2,059 (71.0%)	1,419 (68.9%)	640 (31.1%)		27 (4.2%)	611 (95.5%)	2 (0.3%)	
≥65		542 (18.7%)	437 (80.6%)	105 (19.4%)		8 (7.6%)	95 (90.5%)	2 (1.9%)	
Vaccination status					0.3				0.6
UB-612	non-UB-612								
2	0	103 (3.6%)	71 (68.9%)	32 (31.1%)		0 (0.0%)	32 (100%)	0 (0.0%)	
3	0	212 (7.3%)	153 (72.2%)	59 (27.8%)		4 (6.8%)	55 (93.2%)	0 (0.0%)	
2	≧1	1,569 (54.1%)	1,096 (69.9%)	473 (30.1%)		18 (3.8%)	453 (95.8%)	2 (0.5%)	
3	≧1	1,016 (35.0%)	745 (73.3%)	271 (26.7%)		15 (5.5%)	254 (93.7%)	2 (0.7%)	

[a]Pearson's chi-squared test.
[b]Fisher's exact test.

By estimation, the $pVNT_{50}$ for combating CH.1.1 variant could be 2- to 7-fold lower than that against XBB.1.5 (Figure 4). These findings suggest a likelihood of increased neutralizing antibody escape with the progression of viral evolution. As for subvariants beyond BA.5 and XBB.1.5, the strength of antibody titers across all vaccine platforms, observed at their peak 2–4 weeks post-booster, has been significantly diminished. This could render VNT_{50} or $pVNT_{50}$ measures less relevant at ≥6 or 10 months after booster vaccination. In such cases, longer-lasting T cell immunity becomes crucial in providing protection against breakthrough infection, reinfection, and severe cases.

Post-booster (third dose) cellular IFN-γ and cytotoxic T cell responses

The phase-2 UB-612 booster vaccination, when stimulated by the antigen pool of Th/CTL+RBD, has demonstrated the ability to elicit potent and enduring Th1-oriented IFN-γ^+-ELISpot responses (spot-forming unit [SFU]/10^6 peripheral blood mononuclear cell [PBMC]). Specifically, the responses were measured at 374/261/444 at the peak post-2nd dose/pre-boost/peak post-booster stages, respectively (Figure S3A). This T cell immunity maintained robustness at 70% (261 vs. 374 SFU/10^6 PBMC), persisting 6–8 months post-second dose (pre-boost). Furthermore, upon memory recall via booster vaccination, the response surged to a peak of 444 SFU/10^6 PBMC at 14 days post-booster.

Alongside these findings, there was a notable presence of cytotoxic CD107a$^+$-GranzymeB$^+$ CD8$^+$ T cells, stimulated by the antigen pool of Th/CTL+RBD. These cells were observed at frequencies of 3.6%, 1.8%, and 1.8% at the peak post-second dose, pre-boost, and peak 14 days after the booster dose, respectively (Figure S3B).

Unlike the current mRNA and adeno-vectored vaccine platforms, UB-612 vaccination elicited robust IFN-γ^+-T cell ELISpot responses, as demonstrated in Figure S3C. These levels of response were notably distinct, with the currently authorized vaccine platforms generally yielding lower levels even after a second booster shot.

Moreover, in T cell response data collected from participants in the observational study (comprising 4 infected and 3 non-infected individuals) who received the 3-dose UB-612 regimen and reported no instances of moderate-severe disease, the vaccine's protective effect against severe COVID-19 outcomes demonstrates a strong correlation with IFN-γ^+ and CD107a$^+$-GranzymeB$^+$ cytotoxic CD8 T cells (Figures 5A and 5B), but not with IFN-γ^+-CD4 T cells (Figure 5C), as indicated by the Spearman correlation coefficients of −0.728, −0.721, and −0.218, respectively. Notably, no relationship between disease severity and the decline in viral-neutralizing antibody titers over time (both VNT_{50} and $pVNT_{50}$) could be established, likely due to the short-lived nature of vaccine-induced humoral immunity.

DISCUSSION

The protective effects of UB-612 vaccination, administered with 2 or 3 doses, were assessed 6 months after Omicron outbreak, which equates to a time span of 10–12 months post-booster, or 14–18 months post-second dose, reflecting a pronounced and lasting impact of ≥12 months (with no reported cases of COVID-19 H-ICU). This demonstrates a notable level of clinical significance. In contrast, the effectiveness or efficacy

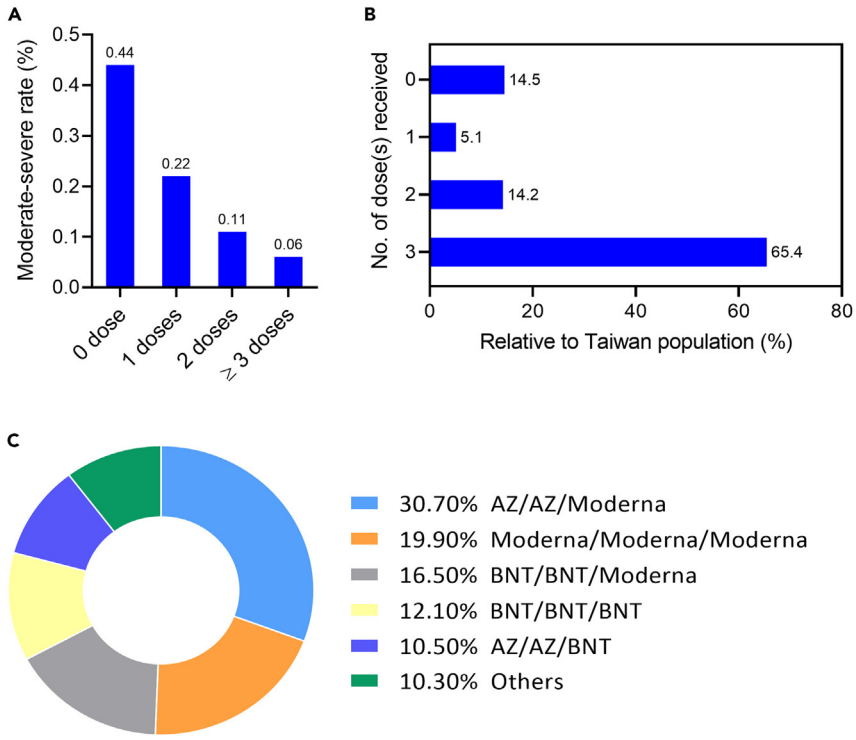

Figure 3. COVID-19 moderate-severe rate and vaccine coverage in Taiwan reported September 30, 2022

On November 18, 2022, Taiwan Central Epidemic Command Center (CECC) of Nation Health Command Center disclosed the moderate-severe rates and EUA-authorized vaccine coverage rates in Taiwan local populations for the period from March 22, 2022 through September 30, 2022, the high time associated with massive surge of Omicron BA.2 and BA.5 infection. The results were adapted from the website of the Taiwan Overseas Community Affairs Council[42], sourced from Central Epidemic Command Center, CECC). September 30 is also the critical time point for evaluation of UB-612 vaccine's protection effects ≥10 months after booster 3rd dose or ≥14 months after the 2nd dose, i.e., 6 months after Omicron outbreak.

(A) Reported on September 30, 2022, the rates of COVID-19 moderate-severe disease (hospitalization and ICU admission) in Taiwan local populations are in an inverse dose number-dependent fashion: 0.44% (14,989/3,374,682) for the unvaccinated, 0.22% (2,583/1,183,209) for those on one dose, 0.11% (3,708/3,288,150) for those on 2 doses, or 0.06% (9,696/16,087,441) for those on ≥3 doses.

(B) The vaccine coverage of 64.5% reported on September 30, 2022.

(C) The vaccine composition of population received 3 doses COVID-19 vaccine in Taiwan, which revealed a mix pool of homologous and heterologous vaccination.

of conventional vaccines is typically evaluated within 30–90 days or up to 6 months post-vaccination.[47,48] It should be noted first that the measures of the descriptive UB-612's protective effect are not intended to be directly comparable to those used for assessing the effectiveness or efficacy of currently approved vaccines.

The data for the overall population of Taiwan, which includes either unvaccinated individuals (0 dose) or those who received EUA-authorized Spike-only vaccines (1, 2, or ≥3 doses), revealed H-ICU case rates ranging from 0.44% to 0.06% (Figure 3A). This suggests that unvaccinated individuals were more susceptible to contracting severe cases of COVID-19, highlighting the additional protective benefit provided by booster shot(s) in combating severe infection. This booster benefit aligns with previous reports indicating that booster doses offer better protection against hospitalization compared to not receiving a booster.[49]

The enduring absence of H-ICU cases for over 12 months (with a 0% rate of moderate-severe disease) can be attributed to the potent and long-lasting T cell immunity generated by UB-612 vaccination (Figure S3). This level of protection appears to be comparable to the immunity conferred by natural infection. Specifically, vaccine-naive natural immunity following a post-Omicron BA.1 infection demonstrates a prolonged and robust safeguarding against severe disease, with a rate of 88.9% maintained for at least 40 weeks[50] Furthermore, this level of protection by infection immunity exhibits a very gradual decline over time.

Furthermore, UB-612's protective effects lasting over 12 months, with 0% occurrence of moderate-severe disease and 27.8% incidence of symptomatic infection, implicate a protective similarity to that reported through hybrid immunity.[51] In comparison to infection-induced immunity, hybrid immunity exhibited higher effectiveness against hospital admission and severe disease, with rates of 97.4% versus 76.4%. Additionally, protection against reinfection was 41.8% versus 24.7% at 12 months after primary vaccination. Notably, similar rates of protection effectiveness were observed for hybrid immunity after receiving a booster dose. It is evident that hybrid immunity offers superior protection compared to immunity from natural infection.

Table 3. Infection rates of COVID-19 observed on two specific dates in populations receiving EUA-vaccines in Taiwan

Observation time point	Date	Total samples	Cases	Infection rate
A. Infection rate in study participants receiving UB-612 vaccine doses[a]				
6 months after 3rd dose (6 weeks after Omicron outbreak)	05/11/2022	337	4	1.2%
10 months after 3rd dose (6 months after Omicron outbreak)	09/30/2022	212	59	27.8%
B. Infection rate in overall Taiwan population[b]				
Observation time point	Date	Taiwan population	Cases	Infection rate
6 weeks after Omicron outbreak	05/11/2022	23,198,133	505,455	2.2%
6 months after Omicron outbreak	09/30/2022		6,461,400	27.9%

EUA-authorized vaccines and the unvaccinated. Data from Taiwan Central Epidemic Command Center (CECC) of Nation Health Command Center and the websites of National Center for High-performance Computing (NCHC), Taiwan [ref. 43]; the website of Department of Household Registration, Taiwan [ref. 44].
[a]For UB-612 booster, infection rates on the median date after 6 months post-booster (05/11/2022) and 10 months post-booster (09/30/2022, the date 6 months after Omicron outbreak).
[b]Infection rates observed on the same two dates for the populations in Taiwan receiving.

By targeting both Spike and non-Spike proteins, UB-612 vaccine-induced immunity may closely resemble the immunity generated by natural infection, especially when compared to vaccines that rely solely on the Spike protein as a single immunogen. Furthermore, focusing on conserved epitopes, particularly those found on non-Spike proteins, is crucial in mitigating the impact of rapid viral mutations and is likely to be a cornerstone for achieving high protection against severe disease.[32–37] A hybrid immunity involving infection and UB-612 vaccination could potentially offer robust protection against severe reinfection.

Throughout the course of viral evolution, a worrisome pattern emerges in viral-neutralizing antibody titers, exemplified by cases like XBB.1.5 versus BA.5 (exhibiting a 10-fold decrease) and CH.1.1 versus XBB.1.5 (showing a 2- to 7-fold drop), as observed in our current data (Figure 4) and corroborated by other reports.[9–12,45,46] With a growing number of reinfections and an escalating incidence of Omicron-to-Omicron reinfections occurring within a shorter time frame compared to pre-Omicron variants of concern,[52] the combination of extraordinary mutability (extending beyond EG.5 and BA.2.86 variants) and the relatively short-lived immune antibody response (fading rapidly beyond detectable levels) indicates that the viral-neutralizing titer (both VNT_{50} and $pVNT_{50}$) is progressively becoming a less relevant parameter for immunity. In contrast, memory T cell immunity (Figure 5) appears to play a more crucial role in ensuring long-term control over hospitalization, severe disease, and reinfection.

There are notable distinctions in the design of vaccines and the immune responses elicited by boosters between the currently approved vaccine platforms and the UB-612 vaccine. The former relies on a full-length Spike protein as the sole immunogen, whereas UB-612 targets the monomeric S1-RBD-focused subunit protein to induce potent viral-neutralizing antibodies.[39,40] Additionally, UB-612 targets multiple conserved, non-mutable helper CD4 (Th) and cytotoxic CD8 (CTL) T cell epitopes on both the Spike (S2x3) and non-Spike (M and N) proteins (Table S2). This approach holds the potential to generate a robust and enduring memory T cell immunity with broad recognition capabilities.

While non-Spike proteins (E, M, N) play a crucial role in eliciting host IFN and memory T cell responses,[32–37] they are also susceptible to mutation (Table S1). It is important to note that mutations on both Spike and non-Spike proteins can potentially result in T cell evasion.[53,54] Given that the group of non-Spike proteins, rather than the Spike protein alone, has been conclusively shown to influence the severity and mortality of COVID-19,[27,31] the emergence of highly dangerous variants capable of systemic infection could be facilitated through gain-of-function genetic manipulation.[26–30] Therefore, a robust defense against established infections would primarily rely on killer CD8 T cells, as opposed to neutralizing antibodies which may be easily outsmarted by the ever-evolving SARS escape variants.

It is worth noting that the observed correlation between the UB-612 vaccine's protective effect against COVID disease severity and the presence of IFN-γ^+ and CD107a$^+$-GranzymeB$^+$ cytotoxic CD8$^+$ T cells (Figures 5A and 5B) underscores the critical role of memory T cell responses. These responses are essential in triggering an immediate IFN reaction and establishing lasting immunity for viral clearance through the activation of killer CD8$^+$ lymphocytes.[32–37]

Furthermore, long COVID has been long thought to be linked to the persistence of viral reservoirs/antigen Spike proteins in tissues[55–59] and in non-classical monocytes that perpetuates inflammation.[56,57] Viral reservoirs not completely cleared by the current Spike-only vaccines may contribute to the persistence of inflammation. Given that long COVID is believed to be linked to a decrease in IFN-γ-producing CD8$^+$ T cells,[60] bolstering T cell immunity to facilitate the clearance of residual systemic infection (such as sustained viral reservoirs) emerges as a prudent strategy for preventing long COVID.

The concerns regarding the unpredictable viral mutations and the potential for a new pandemic catastrophe highlight the crucial and pressing need to integrate multiple conserved, non-mutable T cell epitopes in the development of advanced next-generation COVID-19 vaccines. It is worth noting that robust memory T cell immunity has the potential to provide protection against SARS-CoV-2 infection even in the absence of neutralizing antibodies.[61,62] Ultimately, a potent cytotoxic CD8$^+$ T cell response may serve as the linchpin in regulating the severity of the infection (Figures 5 and S3).

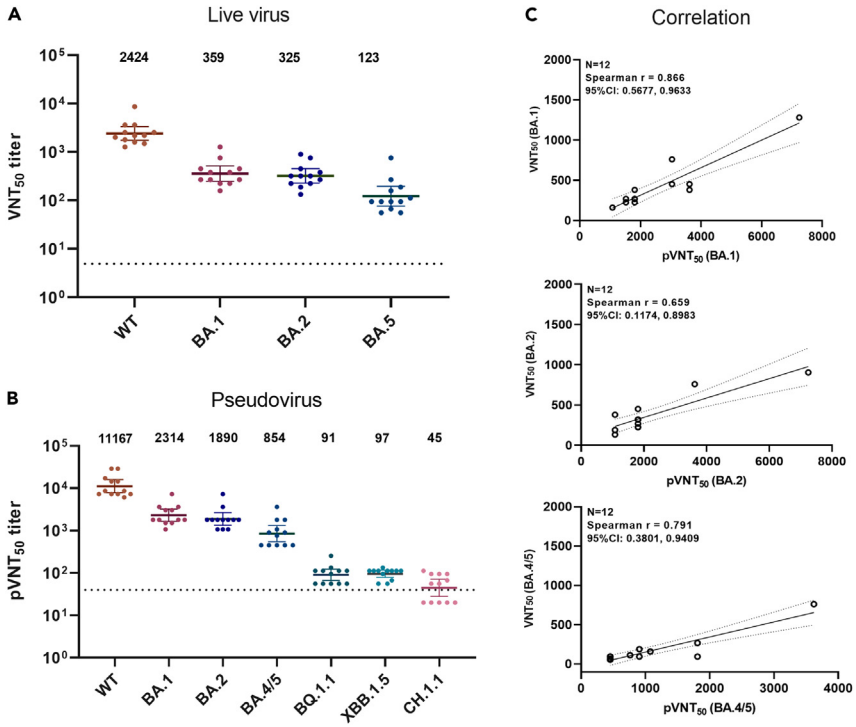

Figure 4. Viral-neutralization antibody titers (VNT_{50} and $pVNT_{50}$) and functional correlations between live virus and pseudovirus

(A and B) In the phase-2 booster extensional trial, serum samples at 14 days poster-booster from 12 study participants (aged 18–85 years) were collected for (A) live virus-neutralization assay (VNT_{50}) against Wuhan wild-type strain (WT) and Omicron variants as indicated, and for (B) pseudovirus-neutralization assay ($pVNT_{50}$).

(C) The correlations between two viral-neutralizing titer assays against pseudovirus ($pVNT_{50}$) and live virus (VNT_{50}) are explored. The correlation coefficients were evaluated by Spearman r with 95% CI.

There is an emerging trend toward the development of a universal mRNA T cell vaccine, known as BNT162b4, which targets conserved epitopes on non-Spike proteins.[31,63] This vaccine aims to broaden the scope of T cell immunity, as intended in the phase-1 trial (NCT05541861). Additionally, when used in combination with the BNT162b2 B5-containing bivalent vaccine, it aims to extend the duration of antibodies against severe disease and hospitalization. Another mRNA vaccine, GRT-R910, based on self-amplifying mRNA (samRNA), also targets conserved epitopes on non-Spike proteins to provide broader and more durable immunity coverage. This vaccine is currently being investigated in a phase-1 trial (NCT05148962), in conjunction with the modified ancestral D614G vaccine that targets the full-length Spike protein.[64,65] The clinical development of these universal mRNA T cell vaccines lends support to the concept of creating a pan-variant UB-612 multitope vaccine, a project that we initiated in early 2020 at the onset of the COVID-19 outbreak.

Conceivably, the unique design of pan-SARS-CoV-2 UB-612 multitope vaccine (Table S2) by itself may lend a support to the finding of pronounced and long-lasting protection against COVID-19 moderate-severe disease (Table 2). And, as shown in an ongoing phase-3 heterologous booster trial (NCT05293665),[66] UB-612 may play a significant role to work as an effective primer and heterologous booster candidate. This is supported by its ability to enhance the seroconversion rate and viral-neutralizing titer against the BA.5 variant for currently approved Spike-only vaccine platforms (Table S6).[67] Understanding the underlying mechanisms of UB-612's protective effects, in face of ever-emerging and higher-contagious variants, requires in-depth clinical investigations, including the impact on long COVID.

Limitations of the study

There are several limitations to consider regarding the e-questionnaire survey utilized in this study. Firstly, it is important to acknowledge the potential for bias due to recall errors or misreporting, even though we took measures to exclude participants who made mistakes in reporting the chronological order of their immunization schedule. Secondly, during the Omicron outbreak, government health agencies directed volunteers to receive only EUA-approved vaccines. This presented challenges in obtaining a large and representative sample size, especially for groups who had completed only the primary UB-612 series or received a homologous booster. Thirdly, since this was a questionnaire-based survey, reported COVID-19 cases were confined to participants who had received a "COVID-19 Home Isolation Notice." This notice could only be obtained if they had either tested positive and been reported by healthcare providers or self-reported to local authorities. As a result, the number of cases observed in this study may have been underestimated.

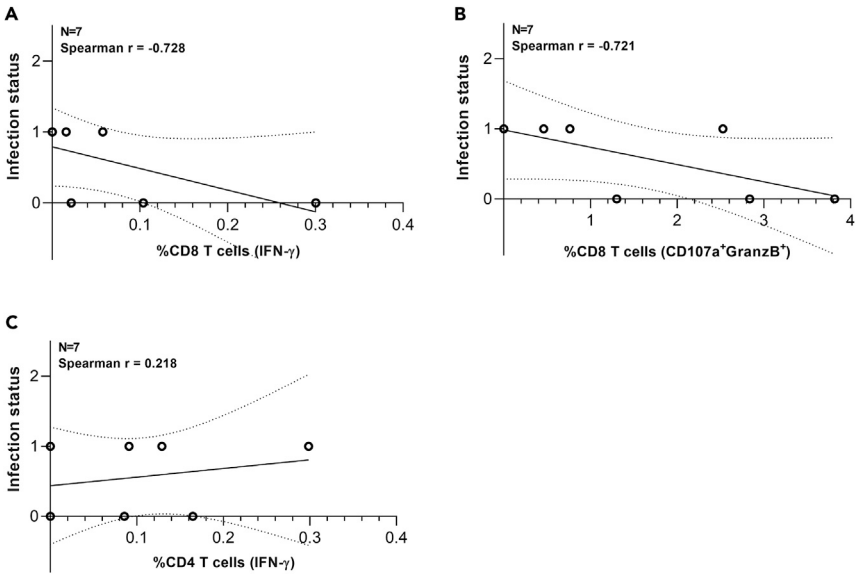

Figure 5. Cytotoxic CD8$^+$ T cell responses induced by UB-612 booster vaccination correlate well with infection status

(A–C) In 7 of the observational study participants who stayed clean on UB-612 course of primary and homologous booster (4 were infected and 3 not infected), their PBMCs collected at 2 weeks post-booster were stimulated with RBD+Th/CTL epitope peptide pool and memory T cell immunity were expressed as (A) IFN-γ^+ %CD8 T cells, (B) CD107a$^+$-GranzymeB$^+$ %CD8 T cells and (C) IFN-γ^+ %CD4 T cells. The T cell responses were analyzed for their correlation (Spearman r) with infection status observed ≥6 months post-booster, with infected = 1 or uninfected = 0. The results indicate that the infection severity was well correlated with CD8$^+$ T cells (IFN-γ^+ or CD107a$^+$-GranzymeB$^+$), but not with CD4$^+$ T cells (IFN-γ^+).

STAR★METHODS

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

C.Y.W. and W.-J.P. were responsible for vaccine development including study protocol design and implementation of the clinical studies. C.Y.W., W.-J.P., and B.-S.K. were responsible for interpretation of the clinical data. Y.-H.L., Y.-H.H., Y.-H.P., H.-C.C., and L.-F.F. were responsible for data acquisition, analysis, and preparation of respective report. Y.-T.Y. was responsible for management of laboratory testing and data preparation. C.Y.W., Y.-H.L., Y.-H.P., and B.-S.K. had full access to and verified all the data in the study and take responsibility for the integrity and accuracy of the data analysis. B.-S.K. and C.Y.W. drafted, prepared, and reviewed the manuscript. All authors reviewed and approved the final version of the manuscript. C.Y.W. had final responsibility for the decision to submit for publication.

DECLARATION OF INTERESTS

C.Y.W. is a co-founder and board member of UBI, United BioPharma, and UBI Asia and named as an inventor on several patent applications filed covering COVID vaccine development. W.-J.P. is also named as a co-inventor on related patent applications. C.Y.W., W.-J.P., B.-S.K., Y.-H.L., Y.-H.H., Y.-H.P., Y.-T.Y., H.-C.C., and L.-F.F. are employees within the UBI group.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-human IFN-γ antibody	Beckman Coulter	Cat# IM2717U; RRID: AB_2892135
Anti-human IL-2 antibody	Biolegend	Cat# 500304; RRID:AB_315091
Anti-human IL-4 antibody	Biolegend	Cat# 500834; RRID:AB_2616758
Anti-human CD107a antibody	Biolegend	Cat# 328630; RRID:AB_2562109
Anti-human Granzyme B antibody	Biolegend	Cat# 396410; RRID:AB_2801079
Bacterial and virus strains		
SARS-CoV-2, WT variant	Taiwan Centers for Disease Control	SARS-CoV-2-Taiwan-CDC#4
SARS-CoV-2, Omicron variant (BA.1)	Taiwan Centers for Disease Control	SARS-CoV-2-Taiwan-CDC#16804
SARS-CoV-2, Omicron variant (BA.2)	Taiwan Centers for Disease Control	SARS-CoV-2-Taiwan-CDC#19380
SARS-CoV-2, Omicron variant (BA.5)	Taiwan Centers for Disease Control	SARS-CoV-2-Taiwan-CDC#689423
Critical commercial assays		
Human IFN-γ/IL-4 FluoroSpotPLUS kit	MABTECH	Cat# FSP-0116
Experimental models: Cell lines		
Human: HEK-293T/17	American Type Culture Collection	ATCC Cat# CRL-11268TM; RRID:CVCL_UE07
African green monkey: Vero-E6	American Type Culture Collection	ATCC Cat# CRL-1586; RRID:CVCL_XD71
Software and algorithms		
FACSCanto II flow cytometry	BD Biosciences	https://www.bdbiosciences.com/en-us/products/instruments/flow-cytometers/clinical-cell-analyzers/facscanto/bd-facscanto-ii-system-software-full-v-3-0-including-dongle-win-7-32-bit-os.659529 RRID:SCR_018056
RStudio	RStudio, Inc.	https://posit.co/download/rstudio-desktop/ RRID:SCR_000432

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Dr. Chang Yi Wang (cywang@ubiasia.com).

Materials availability

This study did not generate any new materials. All plasmids used in the study were operated by RNAi Technology Platform and Gene Manipulation Core of Academia Sinica in Taiwan.

Data and code availability

- The sequence of variants of SARS-CoV-2 is publicly available at outbreak.info (https://outbreak.info/situation-reports).
- The datapoint of COVID-19 pandemic development in Taiwan is publicly available at Crucial Policies for Combating COVID-19 (https://covid19.mohw.gov.tw/en/sp-timeline0-206.html).
- This study does not report any original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Experimental models

- human subjects.
- Asian.

- Median age: 40 years.
- 1476/2900 (50.9%) males.

Participants

This UB-612 Observational Study (see IRB approved Protocol V-205-Q in supplementary appendix) conducted in Taiwan (October 2022 to March 2023) is a retrospective, questionnaire-based survey, derived from an extension cohort with healthy individuals aged 12–85 years in a Phase-2 trial (protocol V-205, ClinicalTials.gov ID: NCT04773067). A total of 3654 participants who had received UB-612 primary series were identified and invited to join the web-based questionnaire survey (Figure 1). All eligible individuals were initially contacted by phone to ascertain study consent and provided with a hyperlink to access the web-based questionnaire.

METHOD DETAILS

Web-based questionnaire survey

An anonymous survey was included in an SMS message with hyperlink that directed participants to the Viedoc cloud-based platform. The questionnaire provided a disclosure of all the necessary information regarding the survey and a consent form on the first page. Once participants agreed to participate, they were able to continue with the rest of the questionnaire, which required them to provide information about their vaccination schedule, SARS-CoV-2 infection history and severity level. Additionally, any changes and repeatedly filling out of the questionnaire were prohibited after the completion of the survey to maintain the integrity of the data collected.

Vaccination status and history of COVID-19

Two sources were used to obtain information on vaccination status and the history of SARS-CoV-2 infection. First, participants were requested to provide the dates of receiving COVID-19 vaccines regardless of types as recorded on their Vaccination Record Card. Second, the information on SARS-CoV-2 infection was obtained from the "COVID-19 Designated Residence Isolation (Home Isolation) Notice and Right to Petition for Habeas Corpus Relief," which is given to individuals who test positive for COVID-19 through a rapid antigen test or reverse transcription polymerase chain reaction (RT-PCR) and are reported by the medical providers or self-report to the government.

Inclusion and exclusion of responses from study participants

Responses from study participants were reviewed for inclusion based on the completeness of the questionnaire. Invalid response was identified by the presence of one or more of the followings: (1) missing responses, (2) incorrect reporting of dates of vaccination dates and reports of SARS-CoV-2 infection within the phase 2 clinical trials of the UB-612 vaccine, and (3) reporting of vaccination dates that were not in chronological order.

Definitions of vaccination status and COVID-19 severity

The observation period was determined from the date of completing UB-612 primary series to 6 months after the Omicron outbreak in Taiwan, specifically until September 30, 2022, the date the study participants were categorized into five groups based on their vaccination status. These groups include individuals who had received only UB-612 primary 2-dose series, received UB-612 primary series with a homologous booster, received UB-612 primary series with a heterologous booster, received the UB-612 primary series with more than one heterologous vaccines, and who received 3 doses of UB-612 vaccine with at least one heterologous vaccine.

Each participant was required to evaluate their situation based on the given definition to determine the severity level of COVID-19 disease. Asymptomatic cases were classified as individuals with COVID-19 who showed no symptoms. Those who experienced COVID-19 symptoms but did not require hospitalization fell into the mild case category. Moderate and severe cases were defined as individuals with COVID-19 requiring hospitalization and ICU admission, respectively.

Study approval and ethics statement

The Observational Study (V-205-Q) adhered to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use, Good Clinical Practice guidelines, Declaration of Helsinki and all relevant government regulations. The protocol, web-based questionnaire and informed consent form were approved by the Institutional Review Boards (IRB) of the participating medical centers (see Appendices S1 and S2).

It is important to note that this survey posed no more than minimal risk to the subjects involved. Eligible participants were selected from among those who volunteered to take part in the Phase 2 Clinical Trial. Investigators were approved to obtain verbal consent from participants over the phone by IRB during the screening process. Additionally, participants were requested to reaffirm their willingness after receiving the web-based questionnaire with a thorough explanation of the research. It was considered informed consent, if participants agree to participate after reading the information and fill in the questionnaire.

To ensure confidentiality throughout the survey, participants were de-identified by assigning to a coding number and were not required to provide identifiable information in the questionnaire. All data was collected via a cloud-based platform (Viedoc Technologies) and is accessible solely by the principal investigators, study coordinators at the sites, and authorized employees at StatPlus, Inc. (Contract Research Organization; CRO) and United Biomedical Inc., Asia (UBIA, the study sponsor).

Live virus-neutralization assay against wild type, BA.1, BA.2, and BA.5 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 Omicron variant (SARS-CoV-2-Taiwan-CDC#16804, BA.1; SARS-CoV-2-Taiwan-CDC#19380, BA.2; SARS-CoV-2-Taiwan-CDC#689423, BA.5), 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% CO2 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 CO2 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 h. 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% CO2, 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 rated for CPE as having a binary result of "infection" or "no infection". The determination of the specific neutralization titer of the SARS-CoV-2 virus was to measure the neutralizing antibody titer againsttheSARS-CoV-2 virus based on the VNT_{50} titer principle (50% reduction in cytological effects induced by the virus). The virus neutralization titer of a serum was defined as the reciprocal of the highest serum dilutioninwhicha50% reduction in the cytopathic effects is observed and the results are calculated using the Reed and Muench method.

Pseudovirus-neutralization assay against wild-type, BA.1 BA.2, BA.5, BQ.1.1, XBB.1.5, and CH.1.1 variants

The neutralizing antibody titers were measured by a neutralization test 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). The 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% CO2 at 37°C. HEK-293T-ACE2 cells were generated by transduction of VSV-G pseudo-typed 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.1, BA.2, and BA.4/BA.5 variants by changing nucleotides from Wuhan-Hu-1 reference strain (Table S7). 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 h post-transfection, cell debris were removed by centrifugation at 4,000 g for 10 min, 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. Sera were be tested by heating 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 h before adding to the plates with cells. After 1-h 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 h 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 h 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 (NT50 titer) was determined by Reed and Muench method.

T cell responses by ELISpot

T cell response was detected in human peripheral blood mononuclear cells (PBMCs). 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 10 μg/mL (each stimulator) of RBDWT + Th/CTL, Th/CTL, or Th/CTL pool without UBITh1a (CoV2 peptides) and incubated in culture medium alone as negative controls for each plate for 24 h at 37°C with 5% CO_2. All analysis processes were 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)

To evaluate $CD4^+$ and $CD8^+$ T cell responses, intracellular cytokine staining, and flow cytometry was applied. Human PBMCs were stimulated 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, respectively for 6 h at 37°C with 5% CO_2. In the stimulating step, cells were washed and stained with viability dye for 20 min at room temperature, followed by surface stain for 20 min at room temperature, cell fixation and permeabilization with the BD cytofix/cytoperm kit (Catalog # 554714) for 20 min at room temperature, and then intracellular stain for 20 min 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.

QUANTIFICATION AND STATISTICAL ANALYSIS

Categorical variables were presented using counts and percentages, while continuous variables were presented as means with standard deviations, medians, and ranges. For comparing different groups, the Kruskal-Wallis rank-sum test was used to assess non-parametric data. Pearson's Chi-squared test was employed to examine the differences between categorical variables, such as sex, age groups, and obesity. Fisher's exact test was utilized when there were fewer observations. The Spearman's rank correlation was performed to assess the relationship between the live virus neutralization assay (VNT_{50}) vs. pseudovirus neutralization assay ($pVNT_{50}$), VNT_{50} (WT) vs. T cell responses, and infection vs. T cell activity.

ADDITIONAL RESOURCES

The present observational study is derived from an extension cohort with healthy individuals aged 12–85 years in a Phase-2 trial (ClinicalTials.gov ID: NCT04773067). The Observational study protocol V-205-Q is provided in the supplementary appendices. All relevant data are within the manuscript and its supplemental information.