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Safety and Immunogenicity of a PHH-1V Booster Dose after Different Prime Vaccination Schemes against Covid-19: Phase III Clinical Trial Final Results Up To One Year

Silvina Natalini Martínez[1], Rafael Ramos[2,3], Jorge Navarro-Perez[4], Maria Jesus Lopez[5], Maria del Mar Vazquez[5], Jose Molto[6,7], Patricia Munoz[8,9,10], Jose M Echave[11], Jose R Arribas[6,12], Melchor Alvarez[13], Eunate Arana-Arri[14], Fernando Cereto[15], Jordi Calls[16], Susana Otero-Romero[17,18], Francesco Scaglione[19], Laia Bernad[20,21], Raúl Pérez-Caballero[20,21], Julia G Prado[6,20,21], Ignasi Esteban[22], Elena Aurrecoechea[22], Roc Pomarol[22], Montserrat Plana[6,22], Alex Soriano[6,23*]

Affiliation:

[1]Unidad de Investigación de Vacunas, Instituto de Investigación Sanitaria HM, Madrid, Spain

[2]Institut Universitari d'Investigació en Atenció Primària Jordi Gol (IDIAP Jordi Gol), Biomedical Research Institute, Girona (IdIBGi), Catalan Institute of Health, Catalonia, Spain

[3]Department of Medical Sciences, School of Medicine, University of Girona, Girona, Spain

[4]Hospital Clínico Universitario Valencia, Valencia, Spain

[5]Preventive Medicine Unit, Hospital Regional Universitario de Málaga, Málaga, Spain

[6]CIBER Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain

[7]Fundació Lluita contra les Infeccions, Department of Infectious Diseases, Hospital Universitari Germans Trias i Pujol, Badalona, Spain

[8]Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Madrid, Spain

[9]Instituto de Investigación Sanitaria Hospital Gregorio Marañón, Madrid, Spain

[10]CIBER Enfermedades Respiratorias- CIBERES (CB06/06/0058), Madrid Spain

[11]Hospital Universitario Quironsalud, Madrid, Spain

[12]Infectious Diseases Unit, Internal Medicine Department, La Paz University Hospital, IdiPAZ, Madrid, Spain

[13]Hospital Universitario Príncipe de Asturias, Madrid, Spain

[14]Scientific Coordination, Biocruces Bizkaia HRI, Osakidetza, Barakaldo, Spain

[15]Hospital Universitario Quironsalud, Barcelona, Spain

[16]Hospital de Mollet, Mollet, Spain

[17]Preventive Medicine and Epidemiology Department, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain

[18]Multiple Sclerosis Centre of Catalonia, Department of Neurology/Neuroimmunology, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain

[19]ASST Great Metropolitan Niguarda, Milano, Italy

[20]IrsiCaixa AIDS, Can Ruti Campus, Badalona, Spain

[21]Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain

[22]AIDS Research Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain

[23]Hospital Clínic of Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

Abstract

In this phase III, open-label, single-arm, multicenter clinical study we report on the safety, tolerability and immunogenicity of PHH-1V as a booster dose in subjects primary vaccinated against COVID-19 with the BNT162b2, mRNA-1273, ChAdOx1-S, or Ad26.COV2.S vaccines, with or without previous COVID-19 infection. A total of 2661 subjects were included in the study and vaccinated with the PHH-1V vaccine. Most treatment-emergent adverse events (TEAEs) were solicited local

***Corresponding author:**
Alex Soriano, Hospital Clínic of Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

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and systemic reactions of grade 1 (58.70%) or grade 2 (27.58%) intensity - the most frequently reported problems being injection site pain (82.83%), fatigue (31.72%) and headache (31.23%). Additionally, immunogenicity was assessed at baseline and on days 14, 91, 182 and 365 in a subset of 235 primary vaccinated subjects. On day 14, the geometric mean titer (GMT) of neutralizing antibody against SARS-CoV-2 Wuhan and Beta, Delta and Omicron BA.1 variants increased in all primary vaccination subjects, with a geometric mean fold rise (GMFR) of 6.90 (95% CI 4.96-9.58), 12.27 (95% CI 8.52-17.67), 7.24 (95% CI 5.06-10.37) and 17.51 (95% CI 12.28-24.97), respectively. Despite GMT decay after day 14, the titers in all cases remained significantly higher versus baseline for up to one year after PHH-1V booster administration, with GMFR against Beta and Omicron BA.1 variants of over 3 at one year after booster compared to baseline. PHH-1V booster vaccination also elicited significant RBD/spike-specific IFN-γ^+ T-cell responses on day 14. Overall, PHH-1V vaccine was immunogenic and well-tolerated regardless of the previous primary vaccination scheme received, with no reported cases of severe COVID-19 infection throughout the entire study.

Keywords: Booster vaccination; COVID-19; Clinical trial; PHH-1V; SARS-CoV-2; Subunit vaccine

ClinicalTrial.gov registration: NCT05303402

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), remains a global health issue, with a total of 774 million confirmed cases and more than 7 million deaths reported globally as of March 2024 [1]. Although COVID-19 vaccines marketed for human use dramatically reduced hospitalization and mortality [2,3], global vaccine supply is inequitable, and only 32.7% of the people in low-income countries have received at least one dose [4]. Moreover, several studies have reported a decline in the effectiveness of COVID-19 vaccines in preventing symptomatic and severe disease over time [5-8]. For instance, a meta-analysis estimated that effectiveness decreased around 10% for severe disease and around 20% for infection over 5 months [9]. This waning of protection has also been enhanced by the emergence of new variants, characterized by increased immune evasion and transmissibility in vaccinated individuals [10,11]. This situation has underlined the need for novel vaccines for booster immunization to supplement the initial primary vaccination and maintain adequate protection of the population. It has been reported that booster doses enhance immunogenicity and restore protection against the Omicron variant [12-15]. For the winter season of 2023, the European Centre for Disease Prevention and Control (ECDC) and the European Medicines Agency (EMA) recommended additional booster doses with adapted COVID-19 vaccines in people aged 60 years and above, in the immunocompromised, in other vulnerable persons (from 12 years of age) with underlying conditions placing them at higher risk of severe COVID-19, and in pregnant women [16].

Several adapted bivalent and monovalent COVID-19 booster vaccines have been developed to cover the demanding vaccine situation and to immunize primary vaccinated individuals against the emerging variants. PHH-1V (BIMERVAX®; HIPRA S.A.) is a recombinant bivalent heterodimer adjuvanted vaccine for the prevention of COVID-19, prepared as an emulsion for intramuscular administration in individuals 16 years of age and older. The vaccine antigen consists of a fusion heterodimer of two receptor binding domains (RBDs) from the SARS-CoV-2 Beta (B.1.351) and Alpha (B.1.1.7) variants, produced as a single chain recombinant antigen in Chinese Hamster Ovary (CHO) cells and adjuvanted with an oil-in-water emulsion based on squalene (SQBA). Subunit adjuvanted vaccines based on recombinant proteins offer several advantages, since they can be efficiently produced on expression platforms and scaled easily at high yields, making them easier to produce and distribute globally. They are also stable and less reliant on a cold chain for their distribution than other COVID-19 vaccines [17].

A first-in-human phase I/IIa dose-escalation, randomized, double-blind, active-comparator controlled clinical trial in 30 healthy adults demonstrated that two doses of PHH-1V vaccine in a range of 10-40 µg/dose were safe and well tolerated, and induced robust humoral immune responses to different circulating variants of concern at the time of the study, including Alpha (B1.1.7), Beta (B.1.351), Delta (B.1.617.2) and Gamma (P.1) [18]. A multicenter, randomized, active-controlled, double-blind, non-inferiority phase IIb clinical trial also showed that PHH-1V displayed a good safety profile, with fewer reported solicited adverse events compared to the Pfizer/BioNTech BNT162b2 vaccine [19]. In this phase IIb study, PHH-1V vaccine elicited a powerful neutralizing antibody response against SARS-CoV-2 Beta, Delta and Omicron strains, including Omicron XBB.1.5 [20], and these results were statistically superior compared to BNT162b2 vaccine at several timepoints for Beta, Delta and Omicron BA.1, and showed non-inferiority against Omicron XBB.1.5 [19,20]. Moreover, the PHH-1V boost also induced a strong and sustained T-cell response against different SARS-CoV-2 variants [19,20].

Herein we report the results of a phase III, open-label, single-arm, multicenter clinical study in healthy adults

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primary vaccinated against COVID-19 (NCT05303402). This phase III clinical study aimed to assess the safety and reactogenicity of a booster dose of PHH-1V vaccine as a heterologous booster in subjects who had been vaccinated (following the local authorities recognized vaccination scheme) at least 91 days before administration of the study vaccine. Participants who had previously suffered from a non-severe or asymptomatic COVID-19 infection were also included. Immunogenicity was also assessed in a subset of subjects previously vaccinated either with homologous or heterologous schemes with BNT162b2 (Comirnaty; Pfizer/BioNTech), ChAdOx1-S (Vaxzevria; AstraZeneca) or mRNA-1273 (Spikevax; Moderna) vaccines.

Materials and Methods

Study design and participants

The present phase III, open-label, single-arm, multicenter clinical trial was carried out to assess the safety, reactogenicity, tolerability and immunogenicity of a booster vaccination with PHH-1V, a recombinant protein RBD fusion heterodimer vaccine against COVID-19. The study was conducted at 17 centers in Spain and a single center in Italy.

Eligible participants were individuals aged 16 years or older who had a COVID-19 vaccination scheme recognized by the authorities of the country with BNT162b2 (Comirnaty; Pfizer/BioNTech), ChAdOx1-S (Vaxzevria; AstraZeneca), mRNA-1273 (Spikevax; Moderna) or Ad26.COV2-S (Jcovden; Janssen) at least 91 days (preferably a maximum of 240 days) before day 0. The patients were required to provide written informed consent, and agreed not to donate blood, blood products or bone marrow at least three months before and after vaccination. For the safety assessment, subjects completed a primary vaccination schedule with the vaccines mentioned above (including those suffering non-severe COVID-19 after second dose at least 30 days before day 0) or were vaccinated with one dose and suffered non-severe COVID-19 infection (confirmed by RT-qPCR or rapid antigen test) before or after receiving the single dose. For immunogenicity assessment, subjects were primary vaccinated with one of the following vaccination schemes: two doses of ChAdOx1-S, two doses of mRNA-1273 or one dose of ChAdOx1-S combined with an mRNA vaccine and without previous SARS-CoV-2 infection. In subjects aged 16 or 17 years old, only those primary vaccinated with two doses of BNT162b2 with no previous COVID-19 infection were eligible for immunogenicity assessment. Additionally, female subjects of childbearing potential were required to have a negative pregnancy test on the day of vaccination and agreed to use any acceptable contraceptive method from day 0 to 8 weeks after vaccination. Key exclusion criteria included a history of anaphylaxis to any prior vaccine, previous severe SARS-CoV-2 infection, previous immunization with live attenuated vaccines within four weeks before or after receiving any study vaccine, pregnancy or breast-feeding at screening, clinically significant acute illness at screening or within 48 hours prior to study vaccination, surgery requiring hospitalization before vaccination, severe and non-stable psychiatric conditions, and abnormal immune system function.

The study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki and in the International Council for Harmonization (ICH) guidelines for Good Clinical Practice (GCP), and with all applicable local laws and regulations.

The study protocol, informed consent form (ICF) and written information given to the subjects were reviewed and approved by an appropriately constituted Institutional Review Board (IRB) and the Independent Ethics Committees (IECs) from Spain and Italy (IEC Hospital Clínic de Barcelona, and IEC LAZZARO SPALLANZANI, IRCCS). The protocol was also reviewed and approved by the Spanish Agency for Medicinal Products and Medical Devices (AEMPS), and by the Agenzia Italiana del Farmaco (AIFA). A Data Safety Monitoring Board (DSMB) was also available to review safety data at any timepoint during the study. Written informed consent was obtained from all participants before enrolment. The method of obtaining and documenting informed consent and the contents of the consent complied with ICH-GCP and all applicable regulatory requirements. All subject identities were kept confidential though the assignment of a unique subject number.

Trial procedures

This study consisted of a maximum 28-day pre-screening period (by phone) prior to the screening/vaccination visit, and a follow-up period of 182 days in the safety assessment subset and 365 days in the immunogenicity assessment subset. The study visits were scheduled on day 0, day 14, day 91, day 182 and day 365 or the early termination visit (ETV). PHH-1V was supplied in a vial containing 10 doses of 0.5 mL (40 µg) ready to use and stored in a refrigerator at 2-8ºC. All eligible subjects received a PHH-1V booster dose on day 0, administered in a volume of 0.5 mL (40 µg) by intramuscular injection into the deltoid muscle. Participants were also given a hard copy diary on day 0 to record local and systemic solicited reactions within the 7 days after vaccination. Blood samples were obtained for safety and immunogenicity assessments according to protocol schedule (Supplementary Table 1).

Safety assessment

Physical examinations and vital signs were recorded at

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Natalini S, et al., Arch Clin Biomed Res 2024
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each study visit before collecting blood samples, and included pulse rate, blood pressure, body temperature and oxygen saturation. Participants were observed for at least 30 min after each injection to identify any immediate adverse event (AE). Solicited local and systemic reactions were recorded daily by the participants in a subject diary until 7 days post-vaccination. Solicited local reactions included pain, tenderness, erythema / redness and induration / swelling. Solicited systemic reactions included fever, chills, nausea / vomiting, malaise / muscle pain, diarrhea, headache, fatigue and joint pain. All subjects were provided with a subject diary to register all local and systemic reactions from the time of vaccination until 7 days post-vaccination. The diary was collected at the day 14 visit. Treatment-emergent adverse events (TEAEs) including unsolicited local and systemic AEs were reported through day 28 after vaccination. Additionally, serious adverse events (SAEs), including suspected unexpected serious adverse reactions (SUSARs), were collected through the end of the study. Adverse events were coded by preferred term (PT) and system organ class (SOC) using the Medical Dictionary for Regulatory Activities (MedDRA version 26.0). An assessment of intensity was made using the general categorical descriptors outlined in the toxicity grading scale for healthy adult and adolescent subjects enrolled in preventive vaccine clinical studies [21]. Grade 3 and 4 changes from baseline in safety laboratory parameters on day 14 after vaccination, as well as SARS-CoV-2 infections and severe cases of COVID-19, were also reported. SARS-CoV-2 infections were assessed with a rapid antigen test or RT-qPCR following the standard procedures in the health system. Severe cases were considered as any episode of COVID-19 requiring ≥ 24 hours of hospitalization. Severe COVID-19 cases which met seriousness criteria were reported as SAEs.

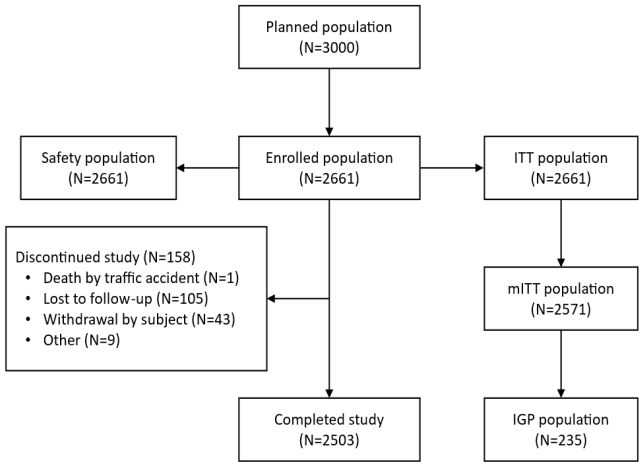

Figure 1: Flow chart of the study.

Abbreviations: IGP: Immunogenicity Population; ITT: Intention-To-Treat; mITT: Modified Intention-To-Treat; N: Number of subjects meeting the criterion.

Characteristics	PHH-1V (N=2661)
Age (years)	
Mean (SD)	34.5 (12.79)
Min-Max	16-85
Age group, n (%)	
≥ 16 to < 18 years	36 (1.35)
≥ 18 to < 65 years	2603 (97.86)
≥ 65 years	22 (0.79)
Sex, n (%)	
Male	1388 (52.16)
Female	1272 (47.80)
Undifferentiated	1 (0.04)
Race, n (%)	
White	2633 (98.95)
Black or African American	8 (0.30)
American Indian or Alaska Native	7 (0.26)
Asian	2 (0.08)
Other	11 (0.41)
Previous COVID-19 infection, n (%)	
≤ 3 months after primary vaccination	707 (26.57)
> 3 months after primary vaccination	66 (2.48)
Prior to the last primary vaccination	377 (14.17)
Missing vaccination date	2 (0.08)
Other missing	4 (0.15)
No	1505 (56.56)
Primary COVID-19 vaccination group, n (%)	
BNT162b2*	197 (7.40)
BNT162b2/ BNT162b2	1550 (58.25)
BNT162b2/ mRNA-1273	13 (0.49)
Ad26.COV2-S*	48 (1.80)
Ad26.COV2-S / BNT162b2	19 (0.71)
Ad26.COV2-S / Ad26.COV2-S	7 (0.26)
Ad26.COV2-S / mRNA-1273	12 (0.45)
mRNA-1273*	90 (3.38)
mRNA-1273/ mRNA-1273	565 (21.23)
mRNA-1273/ ChAdOx1-S	2 (0.08)
ChAdOx1-S*	5 (0.19)
ChAdOx1-S/ BNT162b2	37 (1.39)
ChAdOx1-S/ mRNA-1273	1 (0.04)
ChAdOx1-S/ ChAdOx1-S	112 (4.21)
Others	3 (0.11)
Time elapsed since last primary COVID-19 vaccination dose or infection, n (%)	
≤ 3 months	26 (0.98)
> 3 to ≤ 6 months	721 (27.10)
> 6 to ≤ 12 months	1866 (70.12)
> 12 months	45 (1.69)
Other missing	3 (0.11)

Table 1: Demographic profile and baseline characteristics of participants. Demographics and baseline characteristics were described for the safety population. Percentages were calculated as (%) = n/N*100.

*Subjects with one dose of COVID-19 vaccination and COVID-19 infection (before or after the vaccination).

Abbreviations: COVID-19, corona virus disease 2019; N, the number of subjects in the population; n, the number of subjects meeting the criterion; SD, standard deviation.

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Non-severe COVID-19 cases were considered as AEs, but only confirmed COVID-19 cases occurring after ≥ 14 days post-booster were considered in the exploratory endpoints analysis.

Humoral immunity assays

Neutralizing antibody titers against SARS-CoV-2 Wuhan (original sequence), Beta, Delta and Omicron BA.1 variants were determined by a pseudoviruses-based neutralization assay (PBNA) at HIPRA (Girona, Spain) using an HIV reporter pseudovirus that expresses the S protein of SARS-CoV-2 and luciferase as described previously [22]. Neutralization capacity of the serum samples was calculated by comparing the experimental relative luminescence units (RLUs) calculated from infected cells treated with each serum to the maximal RLUs (maximal infectivity calculated from untreated infected cells) and minimal RLUs (minimal infectivity calculated from uninfected cells), and expressed as percentage neutralization: Neutralization (%) = (RLUmax–RLUexperimental)/(RLUmax–RLUmin) *100. Inhibitory concentration 50 (IC_{50}) values were calculated by plotting and fitting neutralization values and the log of serum dilution to a 4-parameters equation in Prism 9.0.2 (GraphPad Software, USA), and were expressed as the reciprocal concentration for each individual sample and geometric mean titer (GMT) for the descriptive statistics analysis at baseline and on day 14, day 91, day 182 and day 365. The geometrical mean fold rise (GMFR) in neutralizing antibodies titer from baseline to day 14, day 91, day 182 or day 365 was also calculated.

Antibody binding immunity against the SARS-CoV-2 receptor binding domain (RBD) was assessed by Elecsys Anti-SARS-CoV-2 S immunoassay (Roche Diagnostics) according to the instructions of the manufacturer. The GMT values at baseline and on day 14, day 91, day 182 and day 365, and GMFR from baseline to day 14, day 91, day 182 or day 365, were also calculated for the descriptive statistics.

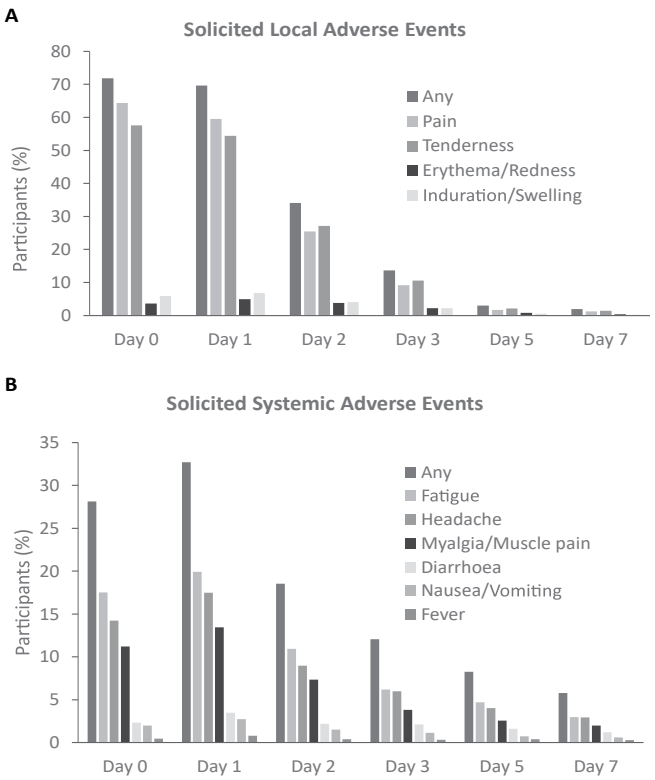

Figure 2: Solicited local and systemic adverse events through Day 7. Solicited local adverse events (A) and solicited systemic adverse events were reported by MedDRA Preferred Term (PT) from Day 0 through Day 7 for the safety population. Data are shown as the percentage of subjects in relation to the safety population (N=2661). If a subject experienced more than one event, the subject is counted once for each type of event. PTs are ordered in decreasing frequency of the total number of subjects with each adverse event.

Abbreviations: N: The number of subjects in the population; PT: Preferred Term

Cellular immunity assays

T-cell mediated immune responses against SARS-CoV-2 were assessed at baseline and on day 14 after vaccination through the measurement of peripheral blood mononuclear cell (PBMC) stimulation by IFN-γ enzyme-linked immune absorbent spot (IFN-γ ELISpot) and intracellular cytokine staining (ICS) testing in a subset of 24 subjects with two doses of ChAdOx1-S vaccine and no COVID-19 infection.

For ELISpot, cryopreserved PBMCs were thawed in FBS and then washed with RPMI followed by 4 h incubation with RPMI complemented with 20% FBS. Cells were counted and plated in 96-wells. The ELISpot plates were previously coated with an anti-IFN-γ capture antibody, using a total of 0.2×10^6 cells per well. Next, PBMCs were stimulated with 6 peptide pools of overlapping SARS-CoV-2 peptides, each encompassing the SARS-CoV-2 region S (2 pools) and RBD (4 pools covering the SARS-CoV-2 Wuhan-Hu-1 strain, and alpha, beta and delta variants), as described in Supplementary Table 2, at a final concentration of 2.5 µg/mL per individual peptide pool. The PBMCs were incubated at a final concentration of 2.5 µg/mL per individual peptide pool. PHA (Sigma-Aldrich; San Luis, MO, USA) was also used as positive control. After overnight incubation, cells were washed 6 times with PBS, incubated with biotin plus anti-human IFN-γ during one hour at room temperature, and washed 6 times with PBS followed by another one-hour incubation step at room temperature with streptavidin. Wells were then incubated with developing solution, followed by 10 minutes at room temperature with 0.05% Tween 20 in PBS 1X and 6 washes with tap water. Spots were counted in a CTL reader system. The total ELISpot responses were reported as the mean value of spot-forming cells (SFCs) per 10^6 PBMCs (SFCs/10^6 PBMCs) upon stimulation with each peptide pool, after subtraction of background.

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Likewise, the frequency of $CD4^+$ and $CD8^+$ T-cells expressing IFN-γ, interleukin-2 (IL-2) and interleukin-4 (IL-4) was assessed by ICS. Cryopreserved PBMCs were thawed in RPMI complemented medium 20% FBS and then washed two times with RPMI 10% FBS. Cells were counted and plated in a 96-well round bottom plate using a total of 0.5×10^6 cells per well. PBMCs were also incubated with the different peptide pools described above in the presence of 2 µg/mL of monoclonal antibodies against human CD28 (clone L293, BD Pharmingen, catalog number 340450) and CD49d (clone L25, BD Pharmingen, catalog number 340976) for 6 hours. During the last four hours of incubation, GolgiPlug (Brefeldin A, BD Cytofix/Cytoperm Plus, BD Bioscience, catalog number 555028) was added to block cytokine transport. After incubation, PBMCs were washed with PBS 1X + 0.5% BSA + 0.1% sodium azide and incubated for 20 minutes with FcR Blocking Reagent (Milteny Biotec, catalog number 130-059-901), then washed and stained for 25 minutes with the Live/Dead probe (LIVE/DEAD fixable near IR, Thermo Fisher Scientific, catalog number L34975) to discriminate dead cells, as well as with surface antigens using the following antibodies: CD3-PerCP (SIK7, BD Biosciences, catalog number 345766), CD4-BV421 (clone RPA-T4, BD Horizon, catalog number 562424), CD8-BV510 (clone SK1, BD Horizon, catalog number 563919). Afterwards, cells were washed twice in PBS 1X + 0.5% BSA + 0.1% sodium azide, fixed and permeabilized with Fix/Perm kit (BD) for intracellular cytokine staining. Cells were then incubated for 25 minutes with FcR Blocking Reagent (Milteny Biotec, catalog number 130-059-901), washed and stained with anti-human antibodies to IFN-γ-APC (clone 27, BD Pharmingen, catalog number 554707), IL-2-PE (clone 5344.111, BD FastImmune, catalog number 340450) and IL-4-PECy7 (clone 8D4-8, BD Pharmingen, catalog number 560672). Finally, stained cells were washed twice with Perm/Wash 1X and fixed in 1% formaldehyde. Cytokine responses were background subtracted. All samples were acquired on a BD FACSCanto II (BD Biosciences) flow cytometer and analyzed using FlowJoTM v.10 software (Tree Star, Ashland, OR, USA).

Outcomes

The primary safety endpoints included the number and percentage of solicited local and systemic reactions through day 7 after vaccination, unsolicited AEs through day 28 after vaccination, grade 3 and 4 changes in safety laboratory parameters from baseline to day 14, and SAEs related to study vaccine through to the end of the study. Secondary immunogenicity endpoints included neutralization titers against SARS-CoV-2 Wuhan, Beta, Delta and Omicron BA.1 variants measured as IC_{50} and reported as reciprocal concentration for each individual sample, GMT for the descriptive statistics analysis at baseline and on day 14, day 91, day 182 and day 365, and GMFR from baseline to day 14, day 91, day 182 or day 365. Additional secondary immunogenicity endpoints were the binding antibody titers measured for each individual sample and GMT for the descriptive statistics analysis at baseline and on day 14, day 91, day 182 and day 365.

Exploratory safety endpoints included the incidence of COVID-19 and the number and percentage of severe COVID-19 cases, both occurring from day 14 after administration of the booster and through to the end of the study. The number and percentage of hospital admissions, intensive care unit (ICU) admissions and noninvasive ventilation procedures associated with COVID-19 were also reported from day 14 to the end of the study. Exploratory immunogenicity endpoints included IFN-γ^+ T-cell and $CD4^+/CD8^+$ T-cell responses to the SARS-CoV-2 S protein measured in re-stimulated PBMCs by ELISpot and ICS, respectively, at baseline and on day 14.

Statistical analysis

No formal sample size calculation was performed for this phase III study. The following analyses of populations were included in the study: intention-to-treat (ITT) population, including all subjects who were enrolled, regardless of subject treatment status in the study; modified ITT (mITT) population, comprising all subjects in the ITT who met the eligibility criteria, received a dose of the vaccine, and had not tested positive for COVID-19 within 14 days of receiving study drug; immunogenicity population (IGP), comprising all subjects in the mITT who had a valid immunogenicity test result before receiving study drug and at least one valid result after dosing; and safety population (SP), comprising all enrolled subjects who received study drug, and were analyzed according to their primary vaccination schemes.

Tabulations were produced for appropriate demographic, baseline, safety and immunogenicity parameters. For categorical variables, summary tabulations of the number and percentage of subjects within each category of the parameter were presented. For continuous variables, the number of subjects, mean, standard deviation (SD), and minimum and maximum values were presented, where appropriate. For the immunogenicity variables, the geometric mean and geometric standard deviations were presented, as appropriate.

Screening demographic characteristics (age, age category, sex, ethnicity, race, height [cm], weight [kg], and body mass index [BMI]), baseline vital signs (systolic and diastolic blood pressure [mmHg], pulse rate [beats per minute], pulse oximetry [%], and body temperature [°C]), primary COVID-19 vaccination group, time elapsed since last dose of primary COVID-19 vaccine (months), and prior COVID-19 infections, were presented using summary statistics. No

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statistical comparisons were performed for any of the baseline characteristics.

A mixed effects model for repeated measures (MMRM) was generated on log-transformed data to measure the neutralization titer against the Wuhan strain and beta, delta and omicron variants as measured by IC_{50} with PBNA. The weighted Least Squared (LS) mean estimates were reported with the associated standard error and 95%CIs. The back-transformed treatment group LS mean estimate for weighted LS means ratio (GMT) were reported with the corresponding 95%CI. Summary statistics for the log10 transformations for each individual sample were calculated based on the log10-transformed titers at baseline and on day 14, day 91, day 182 and day 365, and are presented for the immunogenicity population. To evaluate immunogenicity, total antibody binding antibodies titers measured for each individual sample and GMT for the descriptive statistics analysis at baseline and on days 14, 91, 182 and 365 were analyzed in a similar manner as described above, with GMFR in neutralizing and binding antibodies being assessed using an analysis of variance model (ANOVA model) carried out on log-transformed data. For the cellular immunogenicity analysis, an MMRM of T-cell data was employed, using the angular-transformed proportions as the response variable.

Analyses of AEs were performed for those events that were considered treatment-emergent, with treatment-emergent being defined as any AE with onset on or after the administration of study treatment until 28 days thereafter. TEAEs were presented by maximum intensity (grade 1, 2, 3 and 4) and causal relationship to study drug (pooled as related or not related categories). Solicited local and systemic adverse reactions and unsolicited adverse events after dosing were also presented by maximum intensity and cumulatively across severity levels.

COVID-19 infections and other exploratory safety data are shown as the number of events and the percentage of participants affected in the safety population. An exact 95% Clopper-Pearson CI for the proportion of each endpoint was also presented.

All descriptive statistical analyses were performed using the SAS version 9.4 statistical package, unless stated otherwise. Medical history and adverse events were coded using MedDRA version 26.0 and listed by SOC and PT.

Role of the funding source

This study was sponsored by HIPRA SCIENTIFIC, S.L.U (HIPRA). HIPRA was involved in the study design; in the collection, analysis, and interpretation of data; in writing of the report; and in the decision to submit the paper for publication.

Results

Study participants

Overall, 2661 subjects were screened for this study and were boosted with PHH-1V vaccine. A flow diagram of the study participants is depicted in Figure 1. A total of 158 subjects (5.94%) prematurely discontinued participation in the study. The reasons for premature discontinuation included death (traffic accident), loss to follow-up, withdrawal by subject, and others. All the subjects enrolled in the study were included in the ITT and safety populations. A total of 2571 subjects were included in the mITT population and 235 subjects were included in the IGP population for immunogenicity analysis. The mean study duration for the subjects was 6.4 months (range: 0.03-12.65).

A summary of demographics and baseline characteristics for the safety population is provided in Table 1. Overall, the mean age of the participants was 34.5 years (range: 16-85), 2603 (97.86%) were aged between 18-64 years, 1388 (52.16%) were male, 2633 (98.95%) were Caucasian, and 2242 (84.25%) were not Hispanic or Latino. The mean BMI was 24.69 kg/m^2 (range: 14.86-51.90). At baseline, 1156 subjects (43.44%) had COVID-19 after their primary COVID-19 vaccination, of which 707 (26.57%) experienced COVID-19 $\leq$ 3 months after their primary vaccination, 66 (2.48%) experienced COVID-19 > 3 months after their primary vaccination, and 377 (14.17%) experienced COVID-19 prior to their last primary vaccination. Of the subjects with prior COVID-19 infections, 695 (26.12%) were tested using the rapid antigen test and 477 (17.93%) using PCR. All infections prior to PHH-1V vaccination were non-severe. Additionally, 1550 subjects (58.25%) received BNT162b2/ BNT162b2 as their primary COVID-19 vaccination, 565 (21.23%) received mRNA-1273/ mRNA-1273, and 112 (4.21%) received ChAdOx1-S/ ChAdOx1-S. Most of the subjects (70.12%) received PHH-1V booster > 6 to $\leq$ 12 months after their primary COVID-19 vaccination.

Safety and tolerability

The safety analysis included 2661 subjects who received a dose of study vaccine. The incidence of TEAEs is summarized in Table 2. A total of 7573 TEAEs were reported in 2347 subjects (88.20%). Most TEAEs were of grade 1 (58.70%) or grade 2 (27.58%) intensity. Overall, 22 subjects (0.83%) had TEAEs not related to the administration of PHH-1V, and 2325 (87.37%) had TEAEs related to the administration of PHH-1V. The TEAEs reported in $\geq$ 1.0% of the subjects are summarized in Supplementary Table 3. The most common TEAEs were injection site pain (82.83%), fatigue (31.72%), headache (31.23%) and myalgia (20.74%).

A total of 6861 solicited local and systemic adverse events were reported in 2320 subjects (87.19%), with most being of

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Natalini S, et al., Arch Clin Biomed Res 2024
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grade 1 (59.49%) or grade 2 (26.12%) intensity (Table 2). Of these, 6857 solicited local reactions and systemic events were related to the administration of PHH-1V. The largest reported number of solicited local reactions was on day 0, with 3498 events in 1912 subjects (71.85%), decreasing each day through to day 7 (1.92% of the subjects) (Figure 2A). The most frequently reported solicited local reactions from day 0 to day 7 were pain and tenderness, with 64.37% of the subjects experiencing pain and 57.57% experiencing tenderness on day 0, followed by a decrease to 1.28% and 1.43%, respectively, on day 7. On day 0, the number of solicited systemic events was 1285 in 748 subjects (28.11%), with a peak on day 1 (32.69%) and a subsequent decrease through to day 7 (5.79%) (Figure 2B). The most common solicited systemic events from day 0 through to day 7 were fatigue, headache and muscle pain, with 19.92% of the subjects experiencing fatigue, 17.47% experiencing headache, and 13.45% experiencing muscle pain on day 1, followed by a decrease to 2.97%, 2.93% and 1.99%, respectively, on day 7.

Unsolicited AEs were reported in 538 subjects (20.22%), with most corresponding to grade 1 intensity (16.42% of the subjects) (Table 2). In total, 430 unsolicited AEs were not related to the administration of PHH-1V in 311 subjects (11.69%) and 282 were related to the administration of PHH-1V in 227 subjects (8.53%). Unsolicited adverse events from day 0 through to day 28 in ≥ 1% of the overall subjects are summarized in Supplementary Table 4. The most frequently reported unsolicited AE up to day 28 was COVID-19 infection, with 53 events in 53 subjects (1.99%). Other unsolicited AEs were axillary pain (1.39%), lymphadenopathy (1.24%) and injection site induration (1.16%).

	PHH-1V (N=2661)	
	Events	**Subjects (%)**
Total number of TEAEs	7573	2347 (88.20)
TEAE intensity[1]		
Grade 1	6173	1562 (58.70)
Grade 2	1329	734 (27.58)
Grade 3	68	49 (1.84)
Grade 4	3	2 (0.08)
TEAE relationship to study treatment		
Unrelated[2]	434	22 (0.83)
Related[3]	7139	2325 (87.37)
Total number of TEAEs leading to death†	1	1 (0.04)
Total number of solicited local and systemic AEs*	6861	2320 (87.19)
Intensity of solicited local and systemic AEs[1]		
Grade 1	5577	1583 (59.49)
Grade 2	1226	695 (26.12)
Grade 3	56	41 (1.54)
Grade 4	2	1 (0.04)
Total number of unsolicited AE**	712	538 (20.22)
Intensity of unsolicited adverse events[1]		
Grade 1	596	437 (16.42)
Grade 2	103	89 (3.34)
Grade 3	12	11 (0.41)
Grade 4	1	1 (0.04)
Total number of SAEs‡	22	21 (0.79)
Intensity of SAEs[1]		
Grade 2	6	6 (0.23)
Grade 3	13	12 (0.45)
Grade 4	3	3 (0.11)
Relationship of SAEs to study treatment		
Unrelated[2]	21	20 (0.75)
Related[3]	1	1 (0.04)

Table 2: Summary of Treatment-Emergent Adverse Events (TEAEs). TEAEs were described for the safety population (N=2661). A TEAE was defined as an AE that started on or after the date of administration of study treatment until 28 days thereafter. If AE dates were incomplete and it was not clear whether the AE was treatment-emergent, it was assumed to be treatment-emergent.

[1]If a subject experienced more than one TEAE, the subject is counted once at the most severe or most related event.

[2]Unrelated adverse events are those classified as not related and unlikely related.

[3]Related adverse events are those classified as possibly, probably and related. If an AE has a missing relationship it is assumed to be related to the study treatment for analysis purposes.

*Solicited AEs reported up to day 7. **Unsolicited AEs up to day 28. ‡SAEs up to study end. †Car crash

Abbreviations: AE: Adverse Event; SAE: Serious Adverse Event; N: The Number of Subjects in the Population; TEAE: Treatment-Emergent Adverse Event

Over the entire study period, 22 SAEs were reported in 21 subjects (0.79%) (Table 2). The most frequently reported SAEs were joint dislocation (0.08%) and appendicitis (0.08%), but no SAEs were reported in above 1% of the overall population. One SAE, corresponding to a case of grade 2 pericarditis in a male between 35-40 years old, was considered related due to a temporal relationship to the administration of PHH-1V, and was reported as a SUSAR. The event appeared 14 days after PHH-1V administration and was considered resolved 143 days later. A road traffic accident was reported as fatal SAE unrelated to the administration of PHH-1V.

Vital signs measurements (systolic and diastolic blood pressure, pulse rate, oxygen saturation and temperature) were

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Natalini S, et al., Arch Clin Biomed Res 2024
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within normal clinical values, and no significant changes were observed in the hematology or biochemistry laboratory evaluations among the subjects during the period of the study.

No relevant differences in the safety profile were observed between different primary vaccination schedules, and none of the previous primary vaccinations showed more reactogenicity than others after administration of the PHH-1V booster dose (data not shown). Similarly, subjects with a previous history of COVID-19 had no increase in reactogenicity when receiving PHH-1V, independently of the time elapsed from this previous infection.

Neutralizing and binding antibody responses

Immunogenicity was assessed at baseline and on day 14, day 91, day 182 and day 365 in a subset of 235 subjects vaccinated with two doses of BNT162b2/BNT162b2 (individuals 16-17 years of age; n=13), mRNA-1273/mRNA-

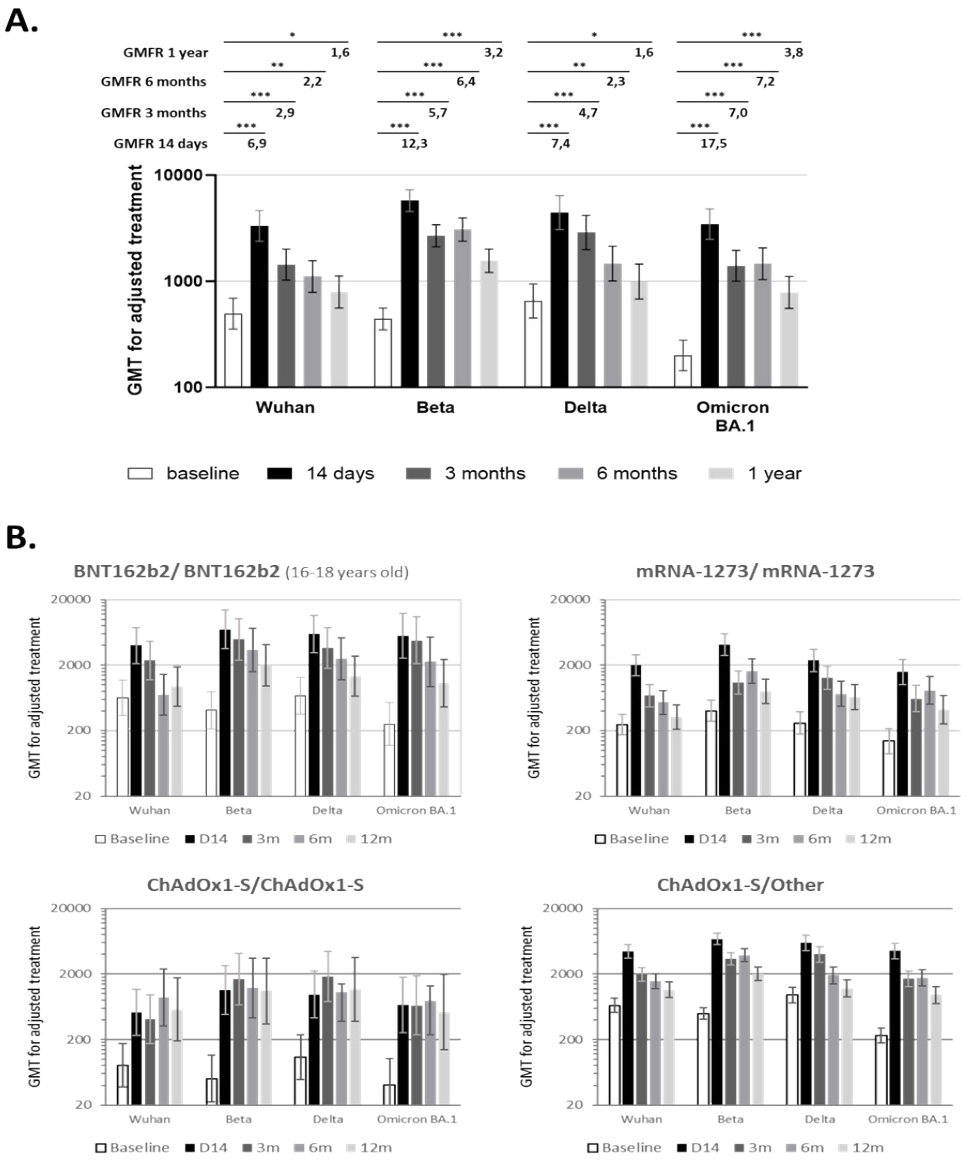

Figure 3: Neutralizing antibody levels against SARS-CoV-2 variants after booster with PHH-1V over time. (A.) Representation of Mean GMT for adjusted treatment with the 95% CI (graphics) and mean GMFR (Upper numbers) from baseline against SARS-CoV-2 Wuhan, Beta, Delta and Omicron BA.1 variants in overall subjects (n= 235) at baseline (White) and Days 14 (Black), 3 months (Dark grey) , 6 months (Grey) and 1 year (Light grey) post-boost. *** p< 0.0001; ** p< 0.001; * p<0,01. (B.) Mean GMT for adjusted treatment with the 95% CI over time for each prime vaccination group. BNT162b2/ BNT162b2 (n=13); mRNA-1273/ mRNA-1273 (n=172); ChAdOx1-S/ChAdOx1-S (n=42) and ChAdOx1-S/other (n=8).

Subjects who reported COVID-19 infections were excluded from the reported day onwards.

Abbreviations: CI: Confidence Interval; GMT: Geometric Mean Titer; GMFR: Geometric Mean Fold Rise

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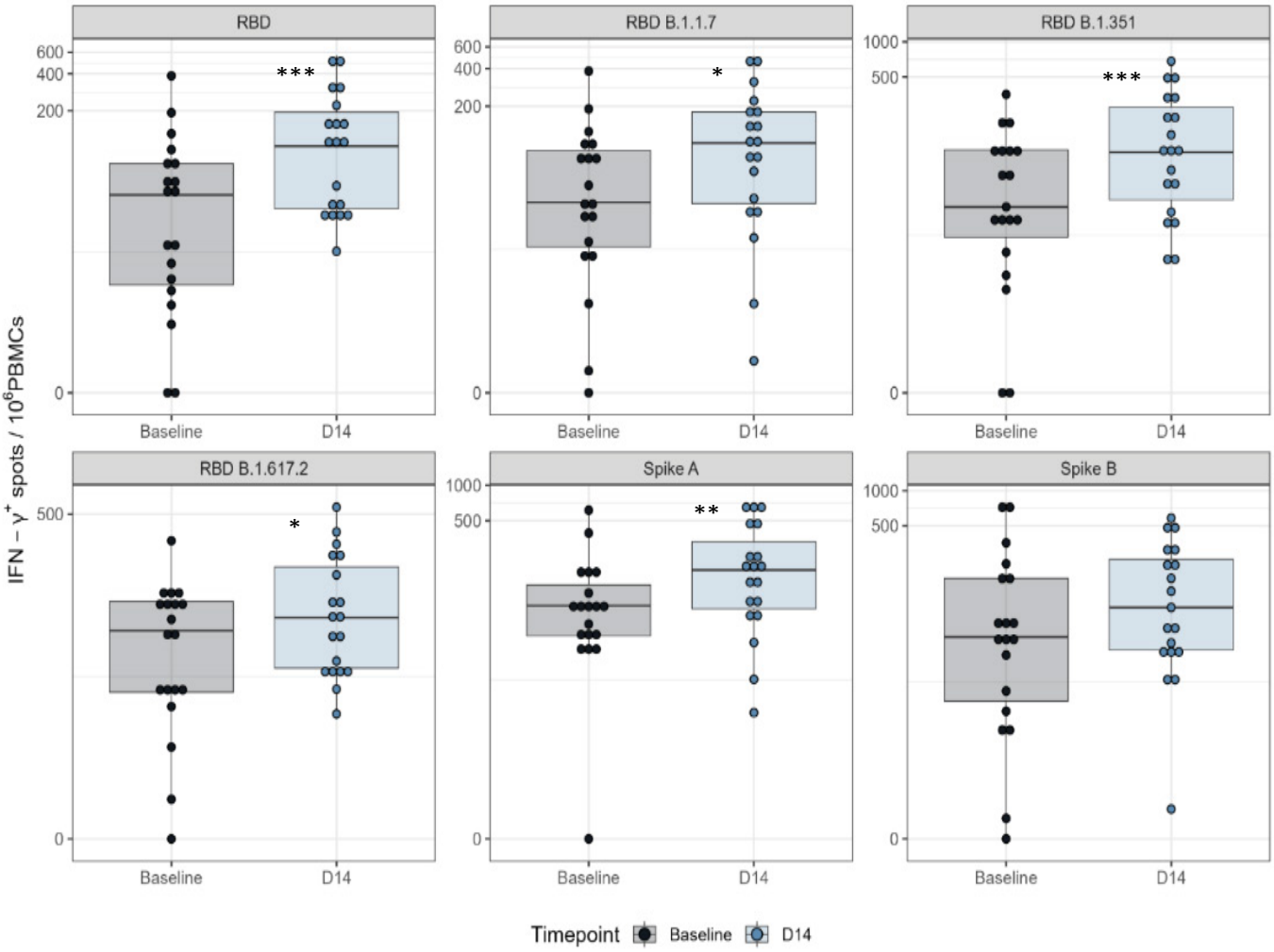

Figure 4: Total IFN-γ producing T cells assessed by ELISpot at baseline and Day 14. Frequencies of IFN-γ responses determined by ELISpot assay in PBMC from subjects immunized with PHH-1V after a primary vaccination with Vaxzevria. PBMC were isolated before the boost immunization (visit 2) and two weeks after boost with PHH-1V (visit 3), stimulated with RBD (Wuhan, RBD alpha, RBD beta and RBD delta) and Spike (SA, SB) peptide pools, and analyzed by IFN-γ-specific ELISpot assay. T-cell responses are expressed as IFN-γ+ spots per 106 PBMCs. Boxes depict the median (solid line) and the interquartile range (IQR), and whiskers expand each box edge 1.5 times the IQR. A logarithmic scale has been used for plotting purposes. *p< 0,05; **p< 0,01; ***p<0,001

Abbreviations: IFN-γ: Interferon Gamma; IQR: Interquartile Range; PBMC: Peripheral Blood Mononuclear Cell; RDB: Receptor Binding Domain for the SARS-CoV-2 Spike Protein (ancestor Wuhan-Hu-1 strain); RDB B.1.1.7 (alpha variant); RDB B.1.351 (beta variant); Spike SA corresponds to 194 spike protein peptide pools overlapping the S1-2016 to S1-2196 region of the Spike Protein; Spike SB corresponds to 168 Spike Protein peptide pools overlapping the S1-2197 to S2-2377 region of the Spike protein.

1273 (n=172) or ChAdOx1-S/ChAdOx1-S (n=42), or a combination of ChAdOx1-S and another brand of vaccine (n=8; 7 were vaccinated with ChAdOx1-S/BNT162b2 and one with mRNA-1273/ChAdOx1-S). The GMT of neutralizing antibodies determined by PBNA at baseline and on day 14, day 91, day 182 and day 365 post-booster for the overall participants independently of the prime vaccination group and GMFR against baseline and on day 14, day 91, day 182 and day 365 post-boost are shown in Table 3 and in Figure 3. To avoid potential bias in immunogenicity assessment, all subjects who reported SARS-CoV-2 infection were excluded from the neutralizing and binding antibody analysis. Overall, GMT for neutralizing antibodies increased on day 14 for all SARS-CoV-2 variants analyzed, with a significant increase compared to the baseline titers, with GMFR 6.90 (95%CI 4.96-9.58) for Wuhan, 12.27 (95%CI 8.52-17.67) for Beta, 7.24 (95%CI 5.06-10.37) for Delta and 17.51 (95%CI 12.28-24.97) for Omicron BA.1. A subsequent decrease in GMT for adjusted treatment over time was observed for all analyzed variants, though in all cases it remained significantly different from baseline over all timepoints up to one year after PHH-1V booster administration (Figure 3A and Table 3). The GMT values for adjusted treatment with the corresponding 95%CI for different prime vaccination groups are shown in Figure

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3B and Supplementary Table 5 for the Wuhan, Beta, Delta and Omicron BA.1 variants. Booster dosing with PHH-1V drove a significant increase in neutralized antibodies on day 14 against all SARS-CoV-2 variants analyzed, regardless of the prime vaccination scheme involved (Supplementary Table 5). The prime vaccination groups showed a decrease in GMT values for adjusted treatment after its induction on day 14, though they still remained numerically higher than at baseline for all variants and prime vaccination groups up to one year after booster vaccination with PHH-1V. The exception was the group vaccinated with ChAdOx1-S and another brand of vaccine (n=8; 7 were vaccinated with ChAdOx1-S/BNT162b2 and one with mRNA-1273/ChAdOx1-S); these 8 participants showed sustained and significant neutralizing antibodies titer values up to one year after booster with PHH-1V compared to baseline for all SARS-CoV-2 variants (Figure 3B and Supplementary Table 5).

Binding antibody responses are summarized in Table 4. Overall, a high binding antibody response was observed on day 14 after the booster in all primary vaccination groups. The GMT (95%CI) for binding antibodies against SARS-CoV-2 RBD on day 14 was 63866.67 (35790.22, 113968.32) for BNT162b2/BNT162b2, 51520.75 (41401.54, 64113.25) for mRNA-1273/mRNA-1273, 18443.18 (13149.13. 25868.71) for ChAdOx1-S/ChAdOx1-S and 13666.18 (6597.12, 28309.99) for ChAdOx1-S / another brand. Furthermore, GMFR (95%CI) for binding antibodies against SARS-CoV-2 on day 14 was 12.98 (5.48, 30.77) for BNT162b2/BNT162b2, 16.82 (10.66, 26.53) for mRNA-1273/mRNA-1273, 14.82 (8.38, 26.23) for ChAdOx1-S/ChAdOx1-S and 30.22 (10.51, 86.90) for ChAdOx1-S / another brand (Table 4). GMT values for binding antibodies gradually declined on day 91, day 182 and day 365, but remained higher than the baseline titers. On day 365, GMT (95%CI) against SARS-CoV-2 was 9390.52 (5085.21, 17340.85), 4257.94 (2940.99, 6164.62), 6388 (2493.64, 16364.29) and 10404.04 (8221.36, 13166.21) for the BNT162b2/BNT162b2, ChAdOx1-S / ChAdOx1-S, ChAdOx1-S / another brand, and Spikevax/Spikevax vaccine groups, respectively; and GMFR (95%CI) was 1.66 (0.66, 4.20), 2.88 (1.54, 5.37), 14.8 (3.51, 62.53) and 2.88 (1.79, 4.65) for the BNT162b2/BNT162b2, ChAdOx1-S / ChAdOx1-S, ChAdOx1-S / another brand, and mRNA-1273/mRNA-1273 vaccine groups, respectively.

T-cell responses

The RBD/Spike-specific IFN-γ^+ T-cell response was analyzed by ELISpot in a subset of 24 subjects immunized with PHH-1V after receiving primary vaccination with ChAdOx1-S. Of these subjects, 19 had valid ELISpot measurements at baseline and on day 14, and were eligible for analysis. Immunization with the PHH-1V vaccine, after primary vaccination with ChAdOx1-S, showed a significant activation of IFN-γ-producing cells after *in vitro* re-stimulation with peptide pools of Spike SA (p=0.0019) and RBD (Wuhan, p=0.0005; alpha, p=0.0121; beta, p=0.0007; delta, p=0.0352) at two weeks post-boost (day 14) compared to the activation observed at baseline

GMT											
	Baseline		Day 14		Day 91		Day 182		Day 365		
	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	
WUHAN	496.03	354.71-693.66	3330.60	2381.71-4657.54	1438.49	1025.91-2017.01	1109.13	785.64-1565.80	791.72	559.23-1120.86	
BETA	440.61	347.83-558.15	5751.34	4540.18-7285.58	2680.30	2106.48-3410.43	3064.77	2384.53-3939.08	1561.17	1209.64-2014.85	
DELTA	650.70	450.15-940.60	4439.06	3070.92-6416.73	2878.70	1986.61-4171.39	1467.59	1006.45-2140.02	994.95	680.67-1454.34	
OMICRON BA.1	199.87	143.57-278.25	3458.55	2484.30-4814.86	1399.37	1001.41-1955.47	1462.59	1037.25-2062.36	785.11	554.87-1110.90	
GMFR											
			Day 14			Day 91			Day 182			Day 365		
			mean	95% CI	p-value	mean	95% CI	p-value	mean	95% CI	p-value	mean	95% CI	p-value
WUHAN			6.90	4.96-9.58	<0.0001	2.93	2.10-4.08	<0.0001	2.21	1.57-3.09	0.0005	1.57	1.11-2.20	0.0319
BETA			12.27	8.52-17.67	<0.0001	5.70	3.95-8.23	<0.0001	6.36	4.39-9.23	<0.0001	3.20	2.20-4.64	<0.0001
DELTA			7.24	5.06-10.37	<0.0001	4.66	3.25-6.68	<0.0001	2.34	1.62-3.37	0.0004	1.57	1.09-2.27	0.0426
OMICRON BA.1			17.51	12.28-24.97	<0.0001	7.00	4.89-10.01	<0.0001	7.17	4.99-10.32	<0.0001	3.81	2.64-5.51	<0.0001

Table 3: Analysis of neutralizing antibody against SARS-CoV-2 variants after a booster dose with PHH-1. Mean GMTs for adjusted treatment and corresponding 95% CI against SARS-CoV-2 Wuhan, Beta, Delta and Omicron BA.1 variants in overall subjects (n= 235) at baseline and Days 14, 91, 182 and 365 post-boost are shown. Mean GMFR calculated between time point GMT for adjusted treatment and baseline GMT with GMFR p-value for odds ratio=1 were shown. Raw data provided as < 20 have been imputed as 20 for the purposes of analysis. Any zero values have been imputed to 10 (half the LLOQ) for analysis purposes. In all timepoints except Day 365/ETV, raw data provided as > 20480 have been imputed as 20480 for the purposes of analysis.

Subjects who reported COVID-19 infections were excluded of this analysis from the report day onwards.

Abbreviations: CI, confidence interval; GMT, geometric mean titer; GMFR, geometric mean fold rise; ETV, early termination visit; LLOQ, lower limit of quantification.

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	BNT162b2/BNT162b2	mRNA-1273/mRNA-1273	ChAdOx1-S/ChAdOx1-S	ChAdOx1-S/ Another
	(N=13)	(N=172)	(N=42)	(N=8)
GMT [95% CI]				
Baseline	4268.34 [2391.94 - 7616.74]	3426.17 [2753.24 - 4263.59]	1079.44 [731.10 - 1678.54]	490.52 [236.79 - 1016.14]
Day 14	63866.67 [35790.22 - 113968.32]	51520.75 [41401.54 - 64113.25]	18443.18 [13149.13 - 25868.71]	13666.18 [6597.12 - 28309.99]
Day 91	31183.96 [17215.09 - 56487.63]	27862.81 [22303.89 - 34807.21]	8631.33 [6084.78 - 12243.64]	11737.91 [5466.09 - 25206.04]
Day 182	14236.33 [7551.76 - 26837.86]	17521.55 [13900.35 - 22086.12]	5806.01 [4025.48 - 8374.09]	11740.62 [4941.62 - 27894.14]
Day 365	9390.52 [5085.21 - 17340.85]	10404.04 [8221.36 - 13166.21]	4257.94 [2940.99 - 6164.62]	6388 [2493.64 - 16364.29]
GMFR [95% CI]				
Day 14	13.23 [5.77 - 30.34]***	15.57 [10.82 - 22.41]***	16.13 [9.77 - 26.60]***	30.25 [10.75 - 85.13]***
Day 91	6.5 [2.82 - 14.97]***	8.61 [5.98 - 12.40]***	7.54 [4.56 - 12.49]***	27.7 [9.72 - 78.92]***
Day 182	3.28 [1.41 - 7.63]*	5.52 [3.82 - 7.96]***	5.14 [3.09 - 8.55]***	28.49 [9.70 - 83.69]***
Day 365	2.00 [0.87 - 4.64]	3.3 [2.28 - 4.76]***	3.76 [2.26 - 6.26]***	16.01 [5.33 - 48.10]***

Table 4: Analysis of binding antibody against SARS-CoV-2 after a booster dose of PHH-1V. Binding antibodies titers were analyzed against SARS-CoV-2 in a subset of subjects primary vaccinated with BNT162b2/ BNT162b2 (N=13), mRNA-1273/mRNA-1273 (N=172), ChAdOx1-S/ChAdOx1-S (N=42) and, ChAdOx1-S/ Another (N=8) at baseline and Days 14, 91, 182 and 364 post-boost. Binding antibodies against the SARS-CoV-2 RBD was assessed by the percentage of subjects having a ≥4-fold increase in the binding antibodies titer 14, 91, 182 and 365 days after booster using the Elecsys Anti-SARS-CoV-2 S immunoassay. A MMRM model was fitted to assess the endpoint on the log10 scale. GMTs, GMFRs and the corresponding 95% CI were estimated using LS Means from MMRM models fitted on the log10 scale and then back-transformed. *p<0.05; **p<0.001; ***p<0.0001.

Raw data provided as > 225000 have been imputed as 225000 for the purposes of analysis.

Subjects who reported COVID-19 infections were excluded of this analysis from the report day onwards.

Abbreviations: CI: Confidence Interval; GMFR: Geometric Mean Fold Rise; GMT: Geometric Mean Titer; LS mean: Least Square Mean; MMRM: Mixed Effects Model for Repeated measures; N: Number of subjects in each primary vaccination group; RBD: Receptor binding domain

(Figure 4). No significant differences were observed between IFN-γ-producing cells elicited at baseline and on day 14 when peripheral blood mononuclear cells were stimulated with Spike SB peptide pool. As the Spike B peptide pool did not contain RBD sequences, an increase in T-cell activation after vaccination was not expected.

Furthermore, the frequency of $CD4^+$ and $CD8^+$ T-cells expressing IFN-γ, IL-2 and IL-4 was assessed by ICS at two weeks post-boost (day 14) in the same subset of 24 subjects. Of these, 15 had valid ICS measurements at baseline and on day 14, and were eligible for analysis. Immunization with PHH-1V, after primary vaccination with ChAdOx1-S, elicited $CD4^+$ and $CD8^+$ T cells expressing cytokines upon stimulation with RBD (Wuhan strain or alpha, beta or delta variants) and Spike (SA or SB) peptides on day 14 post-boost (Supplementary Figures 1 and 2). In particular, PHH-1V vaccination elicited significantly higher $CD4^+$ T cell levels expressing IFN-γ upon stimulation with Wuhan (p=0.0073), alpha (p=0.0191) and beta (p=0.0115) peptide pools; and higher $CD4^+$ T cell levels expressing IL-4 upon stimulation with beta (p=0.0331) and SA (0.0229) peptide pools compared to baseline levels. No significant increases were observed in the activation of $CD8^+$ T-cells (Supplementary Figure 2).

Prevalence of COVID-19 in the vaccinated population

No subjects experienced severe COVID-19, none were hospitalized due to COVID-19, none required noninvasive ventilation, and none were admitted to the ICU due to COVID-19 ≥ 14 days after PHH-1V booster and throughout the duration of the study. There were no deaths associated with COVID-19 after PHH-1V booster and throughout the duration of the study. Overall, 43 subjects (1.62% [95%CI: 1.17, 2.17]) had SARS-CoV-2 infection < 14 days after PHH-1V booster, and 397 non-severe COVID-19 cases in 397 subjects (14.92% [95%CI: 13.59, 16.33]) were reported ≥ 14 days after PHH-1V booster and throughout the duration of the study.

Discussion

The present study describes the results from a phase III, open-label, single-arm, multicenter clinical trial in healthy adults fully vaccinated against COVID-19. The primary objective was to assess the safety and tolerability of PHH-1V as a booster dose in subjects previously vaccinated against COVID-19 with the BNT162b2, ChAdOx1-S, mRNA-1273 or Ad26.COV2-S vaccines, including those that had been

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previously infected by SARS-CoV-2. Many of the vaccines in this trial had been used following the recommendations, though the novelty resides in the fact that the results showed a booster dose of PHH-1V vaccine to be immunogenic for up to one year and safe in individuals primary vaccinated with any of the COVID-19 vaccination schemes recognized by the European authorities during the study period.

This study included 2661 participants aged 16-85 years (mean age: 34.5 years), with a predominance of non-Hispanic and non-Latino Caucasian subjects. Most of the subjects had not experienced previous COVID-19 infection, and the rest of the participants reported non-severe COVID-19. The most common primary vaccination series were BNT162b2/ BNT162b2 and mRNA-1273/ mRNA-1273, and most subjects received their full primary vaccination schedule 6-12 months ago. Our patient population mimicked the situation in the European Union at the time of the study, since the most frequently received vaccines in the European Union are BNT162b2, followed by mRNA-1273 and ChAdOx1-S [23].

The incidence of adverse events reported after a booster dose with PHH-1V (88.20%) was similar to or even lower than that reported in the phase IIb trial [19]. Reactogenicity was assessed for 7 days after the booster dose with PHH-1V vaccine. PHH-1V was well tolerated and had low reactogenicity in all the primary vaccination groups, with most of the solicited adverse reaction being of grade 1 intensity. The most common solicited local adverse reactions were pain and tenderness, and the most common solicited systemic adverse reactions were fatigue and headache. The safety profile of PHH-1V described in this phase III clinical trial was very similar to the safety profile reported previously in the phase IIb trial [19]. Although the relative frequencies of local and systemic reactions are difficult to compare across studies, the reactogenicity profile of the vaccine was also consistent with that observed after a booster dose with subunit- and RNA-based vaccines [13,24-26]. Indeed, a recent clinical study evaluating the safety of a third dose with the protein subunit vaccines NVX-CoV2373 (Nuvaxovid, Novavax) and SII NVX-CoV2373 (Covovax; Indian Serum Institute) showed the most common solicited local adverse reactions to be pain and tenderness, and the most common solicited systemic adverse reactions were headache and fatigue [27]. No relevant differences in the safety profile of PHH-1V were observed between different primary vaccination schedules. Furthermore, unsolicited adverse events were reported from day 0 through day 28 of the study, and SAE events were collected through to the end of the study. The frequency of unsolicited AEs was low (20.22%), with most being of grade 1 intensity. Similarly, the frequency of SAEs was low regardless of primary vaccination, and most of them were unrelated to PHH-1V administration. One case of grade 2 pericarditis was reported and considered to be the only related SAE due to a temporal relationship with the administration of PHH-1V. Pericarditis was reported as a very rare AE in COVID-19 mRNA vaccinations, especially in young adults and adolescent males [28]. ChAdOx1-S (Vaxzevria; AstraZeneca), Ad26.COV2-S (Jcovden; Janssen) and mRNA-1273 (Spikevax; Moderna) booster vaccines based on the primary vaccination group [15].

Vaccination with a booster dose of a COVID-19 vaccine has been associated with a decreased risk of developing COVID-19-related symptoms, hospitalization, and death [5,29]. Another study in the United States reported a significant increase in effectiveness in preventing COVID-19-associated hospitalizations with a primary COVID-19 vaccine schedule plus booster doses compared to a primary vaccine schedule alone [30]. Our results are in line with these findings, since no subjects died due to COVID-19, none experienced severe COVID-19, and none were hospitalized or required noninvasive ventilation due to COVID-19 or were admitted to the ICU due to COVID-19 ≥ 14 days after PHH-1V booster and throughout the duration of the study. Only 14.92% of the subjects reported non-severe COVID-19 ≥ 14 days after PHH-1V booster and throughout the duration of the study.

Neutralizing antibodies are accepted correlates of COVID-19 vaccine efficacy, since vaccines inducing high neutralizing antibodies titers (such as NVX-CoV2373, mRNA-1273 and BNT162b2) have shown greater vaccine efficacy in clinical trials than those associated with lower titers [31,32]. In our study, neutralizing antibodies levels were assessed at baseline and on day 14, day 91, day 182 and day 365 in a subset of 235 subjects without reported COVID-19 and vaccinated with two doses of BNT162b2/ BNT162b2 (individuals aged 16-17 years), mRNA-1273/ mRNA-1273, ChAdOx1-S/ChAdOx1-S, or a combination of ChAdOx1-S and another brand of vaccine. Overall, PHH-1V vaccine was able to elicit a high increase in neutralizing antibody titers 14 days after the booster vaccination against the Wuhan, Beta, Delta, and Omicron BA.1 SARS-CoV-2 variants compared to the levels recorded at baseline, regardless of the primary vaccination schedule involved. The GMT levels corresponding to neutralizing antibodies against SARS-CoV-2 gradually dropped on days 91, 182 and 365 of the study, but were still significantly higher than the baseline titers. Only the small group of ChAdOx1-S with another brand of vaccine (n=8; 7 vaccinated with ChAdOx1-S/ BNT162b2 and one with mRNA-1273/ChAdOx1-S) did not show this drop in neutralizing antibodies, which may have been due to the small population included or to unreported COVID-19 infection over the study period. Remarkably, a booster vaccination with PHH-1V was able to sustain good neutralizing activity against Omicron BA.1, the most common variant at the time of the start of this trial [33]. Compared to

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the Wuhan-Hu-1 reference genome, the RBD sequence of Omicron BA.1 comprises 15 mutations localized within the binding sites of epitopes targeted by monoclonal antibodies [34]. K417N substitution, present also in the Beta variant, is one of the 15 RBD substitutions in the Omicron variant and is responsible for significant disruption to known monoclonal antibodies [35]. This fact strongly supports the high humoral immunogenicity of the PHH-1V RBD-based candidate against a wide range of potential new mutations, since the PHH-1V antigen comprises key mutations that are also present in the Omicron BA.1 variant, as well as in many other SARS-CoV-2 variants. Of note, all subjects who reported SARS-CoV-2 infection were excluded from the neutralizing antibody analysis, thus eliminating possible bias in GMT measurement due to infection-induced immunogenicity.

A minimum clinically important 1.75-fold difference in GMT between the pre- and post-booster levels has been proposed based on advice by policy makers in the United Kingdom [15]. Following this criterion, the fold increase in neutralizing antibodies on day 14 after the PHH-1V booster was clinically relevant regardless of the primary vaccination series received, with a GMFR of 6.90 against SARS-CoV-2 Wuhan, 12.27 against the beta variant, 7.24 against the delta variant, and 17.51 against the omicron BA.1 variant. Although the neutralizing antibody GMT decreased throughout the study, GMFR remained over 1.75 on day 365 against the beta (3.20; 95%CI: 2.20-4.64) and omicron BA.1 (3.81; 95%CI: 2.64-5.51) variants for most of the primary vaccination group - showing that PHH-1V elicits a long-term (one year) neutralizing response against a variety of SARS-CoV-2 strains, including new emerging variants, at that timepoint. In addition, binding antibodies were assessed at baseline and on day 14, day 91, day 182 and day 365 post-booster vaccination. In general, a high response in binding antibodies and fold rise in binding antibodies was observed 14 days after the PHH-1V booster dose. Even though a steady decrease in binding antibody GMT values was observed on days 91, 182 and 365 after the PHH-1V booster dose, the titers were still higher than at baseline. Globally, these data demonstrate that a booster dose of PHH-1V can increase and maintain a humoral immune response over a long period of time.

While neutralizing antibodies play a primary role in preventing symptomatic COVID-19, the prevention of severe cases is likely influenced by various immune components such as $CD4^+$T cells, $CD8^+$T cells and memory B cells [36-38]. In this respect, the RBD/Spike-specific IFN-γ^+ T-cell response was analyzed on day 14 by ELISpot in a subset of 24 subjects immunized with PHH-1V after primary vaccination with ChAdOx1-S (Vaxzevria; AstraZeneca). Overall, the heterologous boost with the PHH-1V vaccine, after primary immunization with ChAdOx1-S, elicited a significant increase in RBD/Spike-specific IFN-γ^+ T-cell response against different SARS-CoV-2 variants tested compared to baseline. Likewise, $CD4^+$ and $CD8^+$ T cell responses were also analyzed on day 14 by ICS in the same subset of subjects. In conclusion, the heterologous boost with the PHH-1V vaccine, after primary immunization with ChAdOx1-S, elicits T-cell responses presenting a balanced Th1/Th2 profile, with the activation of RBD/Spike specific $CD4^+$ T-cells.

Our study has some limitations. Immunogenicity assessment at 3, 6 and 12 months has only been studied in a subset of 235 subjects. Most of the participants were also primed with mRNA vaccines, and only a small sample had an adenovirus vaccine shot. No analysis was made of neutralizing antibodies against current circulation variants (XBB.1.5, XBB.1.16, EG.5.1, BA.2.86) or of T-cell responses against any Omicron variant. Nevertheless, in a recent publication we reported that PHH-1V induces a long-term immune response of up to 6 months to the XBB.1.5 SARS-CoV-2 variant, and is non-inferior compared with BNT162b2 [20]. Furthermore, neutralizing activity was only analyzed by PBNA and not by a live SARS-CoV-2 neutralization assay. Despite this fact, new validation analyses confirm the validity of PBNA for SARS-CoV-2 [39].

In conclusion, the PHH-1V vaccine (BIMERVAX®, HIPRA) was well tolerated and safe, regardless of the primary vaccination schedule received or of previous SARS-CoV-2 infection. Heterologous booster with PHH-1V induces a broad and long-lasting humoral immune response against different SARS-CoV-2 variants, and could be an interesting strategy for upcoming vaccination campaigns in individuals already immunized with mRNA and/or vector vaccines. The PHH-1V vaccine has received marketing authorization from the EMA as a booster in people aged 16 years and above who have been vaccinated with an mRNA COVID-19 vaccine [40].

Contributors

Veristat was responsible for managing the data and performing the statistical analyses. The authors contributed to the acquisition, analysis and/or interpretation of data. All authors had full access to all the data, revised the manuscript critically for important intellectual content, approved the version to be published, and accepted responsibility for publication.

Declaration of Interests

SNM declares that her institution received payment from HIPRA for conducting this trial, from Pfizer, Sanofi, MSD, GSK, Janssen, AstraZeneca and Moderna for other vaccines trials, and for participation on data safety monitoring boards

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Data Sharing

All data relevant to the study are included in the article or uploaded as supplementary information. Further data are available from the authors upon reasonable request and with permission from HIPRA, S.A.

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