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RESEARCH ARTICLE

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Safety and immunogenicity of PHH-1V booster against SARS-CoV-2 variants, including omicron subvariants: Results from a phase IIb open-label extension study

María Jesús López[a], Maria Del Mar Vazquez[a], Melchor Alvarez-Mon[b], José Ramón Arribas[c,d], Eunate Arana-Arri[e], Patricia Muñoz[f,g,h], Jorge Navarro-Pérez[i], Rafael Ramos[j,k], José Molto[d,l], Susana Otero-Romero[m,n], Elena Aurrecoechea[o], Roc Pomarol[o], Laia Bernad[p,q], Ignasi Esteban[o], Raúl Pérez-Caballero[p,q], Montserrat Plana[d,o], Júlia G. Prado[d,p,q], and Álex Soriano[d,r]

[a]Preventive Medicine Unit, Hospital Regional Universitario de Málaga, Málaga, Spain; [b]Internal Medicine Unit, Hospital Universitario Príncipe de Asturias, Madrid, Spain; [c]Infectious Diseases Unit, Internal Medicine Department, La Paz University Hospital, IdiPAZ, Madrid, Spain; [d]CIBER Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain; [e]Scientific Coordinator, Biocruces Bizkaia Health Research Institute, Osakidetza, Barakaldo, Spain.g. Clinical Microbiology, Infectious Diseases and AIDS Group, Instituto de Investigación Sanitaria Hospital Gregorio Marañon, Madrid, Spain; [f]Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Madrid, Spain; [g]Instituto de Investigación Sanitaria Hospital Gregorio Marañón, Madrid, Spain; [h]CIBER Enfermedades Respiratorias, CIBERES Group (CB06/06/0058), Madrid, Spain; [i]Hospital Clínico Universitario de Valencia, Valencia, Spain; [j]Vascular Health Research Group, Institut Universitari d'Investigació en Atenció Primària Jordi Gol (IDIAP Jordi Gol), Biomedical Research Institute, Girona (IdIBGi), Catalan Institute of Health, Catalonia, Spain; [k]Department of Medical Sciences, School of Medicine, University of Girona, Girona, Spain; [l]Department of Infectious Diseases, Fundació Lluita contra les Infeccions, Hospital Universitari Germans Trias i Pujol, Badalona, Spain; [m]Preventive Medicine and Epidemiology Department, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain; [n]Multiple Sclerosis Centre of Catalonia, Department of Neurology/Neuroimmunology, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain; [o]AIDS Research Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain; [p]Irsicaixa, Badalona, Spain; [q]Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain; [r]Hospital Clínic of Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

ABSTRACT

SARS-CoV-2 vaccination campaigns on current endemic situation would benefit from vaccine alternatives with easy logistics and accessibility, sustained response and cross-reactivity against emerging variants. Herein, safety and immunogenicity of PHH-1V, adjuvanted recombinant RBD-based vaccine, as fourth dose for the most prevalent SARS-CoV-2 variants in Spain in subjects ≥18 years was investigated for 6 months in HIPRA-HH-2 open-label extension study. Subjects received a fourth dose of PHH-1V after either two BNT162b2 doses plus one PHH-1V dose (cohort 1) or three BNT162b2 doses (cohort 2). As regulatory endpoint, neutralization titers were investigated for PHH-1 V as fourth dose vs BNT162b2 as third dose in subjects receiving previous BNT162b2-based regimens. PHH-1 V immunogenicity (GMT) was investigated against Beta, Delta, and Omicron BA.1, BA.4/5 and XBB.1.5 on Days 14, 98 and 182 post-immunization. Two hundred and eighty-eight subjects received PHH-1V. Neutralizing antibodies against Omicron BA.1 at Day 14 significantly increased after the PHH-1V as fourth booster vs the third BNT162b2 booster (GMT ratio 0.43 (95% CI: 0.28; 0.65; p-value < .0001)). PHH-1V fourth booster induced a significant increase in neutralizing titers vs baseline (GMFR on Day 14 [95% CI]: Beta 6.96 [5.23, 9.25]; Delta 6.27 [4.79, 8.22]; Omicron BA.1 9.21 [5.57, 15.21]; Omicron BA.4/5 11.80 [8.29, 16.80]; Omicron XBB.1.5 5.22 [3.97, 6.87]), remaining significantly higher up to 6 months. The most frequent adverse events were injection site pain and fatigue. As conclusion, PHH-1V booster induced sustained humoral and cellular immune response against Beta, Delta variants and cross reactivity against distant Omicron subvariants and could be an appropriate strategy for implementing heterologous vaccination campaigns.

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Introduction

Since the advent of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1] that emerged in Wuhan, China, in 2019,[2,3] huge efforts had been made to develop multiple prophylactic strategies including vaccines. Nevertheless, severe COVID-19 disease accumulates up to 6.96 million deaths worldwide (10th October 2023).[4,5]

At the population level, immunity against SARS-CoV-2 will increase through widespread immunization and infection occurrence.[6] Despite this, on an individual level, the humoral response against new variants is diminished for both SARS-CoV-2 vaccinated and infected individuals.[6] Vaccines are needed that offer broad and long-lasting immunological protection and reduce the incidence of severe disease and related hospitalizations.[7] In addition, the emergence of new variants for SARS-CoV-2, such as Omicron and its sub-variants,[8] requires the adaptation of immunization strategies[9] by implementing effective vaccination regimens, preferably combining heterologous boosters. A wide range of vaccine approaches were developed at an unprecedented speed after the identification of SARS-CoV-2. While viral vector vaccines use

CONTACT Álex Soriano asoriano@clinic.cat Department of Infectious Diseases, Hospital Clínic of Barcelona, Helios Building, First Floor, Desk n° 25, Carrer Villarroel, 170, Barcelona 08036, Spain.
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a modified version of a different virus as a vector to deliver protection,[10] mRNA vaccines use genetically modified RNA to generate a protein that, in turn, elicits a safe immune response.[11] Additionally, there are also adjuvanted protein-based subunit vaccines, such as PHH-1V, which represent a new generation of vaccines that elicit a safe and strong immune response targeted to key parts of the virus with a better reactogenicity profile than mRNA-based vaccines,[12,13] even in persons with a weakened immune system.[10]

Although primary vaccination offers good protection against severe disease, national and international studies using mRNA COVID-19 vaccines have shown a reduction in effectiveness in adults 3–6 months post-vaccination, especially in terms of infection rates.[12] Earlier studies demonstrated a rapid decline and further stabilization of neutralizing antibodies[14] sustained by long-lived B cells directed against SARS-CoV-2 S[15] among patients who recovered from severe COVID-19 or mild COVID-19. These observations suggested that the strong humoral immune response to the infection would not be sustained for long periods, a situation that was confirmed in the ensuing months and years. Similarly, the robust antibody response triggered by mRNA vaccines declined over time.[16] An analysis based on data from 33,418 patients vaccinated with eight SARS-CoV-2 vaccines through different platforms revealed that all eight vaccines lost most of their protective effect against symptomatic infection between 8 and 11 months after the first dose.[17] Consequently, most national vaccine strategies included additional booster doses with either the same vaccine type (homologous booster) or a different vaccine type (heterologous booster) as both approaches have proven to provide appropriate immunogenicity.[12] In fact, booster strategies have demonstrated higher efficacy in reducing the risk of not only COVID-19 infection but also of long COVID-19 or persistent post-COVID manifestations in up to 73% of subjects after receiving three doses.[18] In addition, the presence of adjuvants contributes to the induction and establishment of a sustained immune response, thus enhancing the overall magnitude and durability of immune response in the long-term.[19] Although waning humoral immunity was still occurring following booster immunizations with mRNA vaccines, even after transient improvement of serum antibody responses,[20,21] waning was faster for Omicron BA.1, with most individuals not reaching protective neutralizing antibody levels. In addition, some studies have reported that the waning of vaccine effectiveness against hospitalization was more pronounced in high-risk populations, such as older adults, immunosuppressed patients, and those with comorbidities and was associated with lower initial values.[22]

During the Omicron wave, several subvariants emerged, with greater infectivity and immune evasion and they rapidly replaced the previous Delta variants as the dominant variants. Two Omicron subvariants, BQ.1 and XBB lineages, became rapidly a global public health issue given their ability to escape from therapeutic monoclonal antibodies and herd immunity induced by prior coronavirus disease 2019 (COVID-19) vaccines, boosters, and infection.[23,24]

Subtle difference in terms of the mechanisms by which the different vaccine classes (mRNA-, adenoviral vector- and recombinant protein-based) elicit immune response have emerged.[25,26] Moreover, adjuvanted vaccines induce high levels of protective antibodies, long-lasting immune response of memory cells and a higher degree of cross-immunization than do non-adjuvanted.[19–31] More studies are needed to determine whether these subtle differences between vaccine platforms or heterologous vaccination approaches with new vaccines could be beneficial for specific vaccination groups like elderlies or individuals with immunocompromised conditions that are nowadays the priority groups on COVID-19 vaccination campaigns.

PHH-1V (BIMERVAX®; HIPRA, Spain) is a bivalent dimeric recombinant protein adjuvanted vaccine against SARS-CoV-2 and is based on a heterodimer protein comprising a recombinant receptor-binding domain (RBD) fusion of Spike protein from two SARS-CoV-2 variants, B.1.351 (Beta) and B.1.1.7 (Alpha). PHH-1V is indicated as a booster dose for active immunization to prevent COVID-19 in people aged 16 years or older who have received a COVID-19 mRNA vaccine.[12,32–34] On 30th March 2023, the EMA recommended the approval of PHH-1V as a COVID-19 booster vaccine,[12,25,33,34] on 1st August 2023 it was authorized by the Medicines and Healthcare products Regulatory Agency (MHRA),[35] and on 9th October 2023 the World Health Organization (WHO) included PHH-1V in its list of prequalified vaccines.[36]

Previous clinical data on PHH-1V has shown that it elicits high and long-lasting levels of neutralizing antibodies against all COVID-19 variants studied, as well as a strong cellular immunity response, when used as a heterologous booster in previously vaccinated individuals with mRNA and viral vector vaccines.[13,32–34,37,38] Moreover, booster with PHH-1V has shown a good safety profile and less reactogenicity compared to booster with mRNA-based vaccine comparator.[12,13,38] These features along with a ready-to-use formulation without the need for reconstitution, storage at 2°C to 8°C and a prolonged shelflife mean that PHH-1V is a suitable, next-generation vaccine option for either annual, seasonal, or targeted immunization programs against SARS-CoV-2 to improve the protection of high-risk groups. The favorable reactogenicity profile for PHH-1V may be of relevance in the context of the slowing of the uptake of SARS-CoV-2 booster vaccinations reported in many countries worldwide.[39,40]

Herein we present results from the HIPRA-HH-2 open-label extension study with participants mainly enrolled on HIPRA-HH-2 trial and an additional analysis comprising new viral variants of interest in Spain in the Omicron era. HIPRA-HH-2 (NCT05142553) study was a Phase IIb, randomized, double-blind, controlled, multicentre, non-inferiority clinical trial in 765 participants vaccinated against COVID-19 with BNT162b2 (tozinameran) at least 182 days prior to the administration of PHH-1V ($n = 513$) or BNT162b2 ($n = 252$) as first booster dose in 10 centers in Spain.[13,37] Geometric mean titers (GMT) were studied after first booster dose and results indicated superiority of PHH-1V at Days 98 and 182 compared with BNT162b2 and non-inferiority at Days 14 and 28, depending on the variant evaluated.[13] The overall frequency of adverse events (AEs) was significantly lower ($p < .05$) among subjects who received the PHH-1V booster

versus those receiving BNT162b2, with most AEs in both groups being mild.[13,37] In addition, the PHH-1V vaccine was well tolerated and safe, regardless of vaccination history.[13,34,37]

The aim of this investigation was to evaluate the immunogenicity and safety of PHH-1V (beta/alpha heterodimeric vaccine) when administered as a second booster vaccination against SARS-CoV-2 variants of special interest during a period with a wide range on Omicron variants present in Spain.

Materials and methods

Study design and participants

HIPRA-HH-2 extension, a phase IIb open-label extension study (NCT05142553) that evaluated the safety and immunogenicity of PHH-1V administered as a fourth dose in adult participants (≥18 years), started in September 2022 in 10 centers across Spain. The trial was conducted in accordance with the Declaration of Helsinki, the Good Clinical Practice guidelines, and national regulations. The study protocol was reviewed and approved by the Spanish Agency of Medicines and Medical Devices (AEMPS) as well as Independent Ethics Committee from the Hospital Clínic de Barcelona (HCB/2021/1110).

Study population were adult participants (≥18 years of age) who had completed 6 months on the HIPRA-HH-2 study and fulfilled the inclusion criteria to be enrolled in the extension phase of the study. Inclusion and exclusion criteria for the HIPRA-HH-2 study have been published previously.[13] A fourth dose of PHH-1V was administered between 6 and 12 months to two cohorts of subjects which had received three previous doses of a SARS-CoV-2 vaccine based on different vaccination schemes: participants who had received prime vaccination with two doses of BNT162b2 plus a third dose of PHH-1V as first booster (Cohort 1), and participants who received three doses of BNT162b2, two doses as prime vaccination and one as first booster, (Cohort 2). Additionally, a group of participants from the community who matched the vaccination history of the HIPRA-HH-2 study and fulfilled the inclusion/exclusion criteria were recruited and included in Cohort 2 of the HIPRA-HH-2 extension phase to answer the primary objective (described below). Concomitant medications prohibited during the open-label study included anticoagulants, immunosuppressants and other immune-modifying treatments administered within 2 months before Day 0 and throughout the study.

Written informed consent was obtained from all participants before enrollment.

Objectives

The primary objective of HIPRA-HH-2 extension study, which followed regulatory requirements, was to determine and compare the changes in immunogenicity measured by pseudovirion-based neutralization assay (PBNA) against Omicron BA.1 subvariant at Day 14 post-fourth dose of PHH-1V in Cohort 2 vs BNT162b2 as post-third dose in Cohort 2 from the initial HIPRA-HH-2 study. Secondary objectives were to determine and compare changes in immunogenicity (by PBNA) against Omicron BA.1, Omicron BA.4/5, and Beta and Delta variants at Days 14, 98 and 182 post-fourth dose of PHH-1V versus baseline, T-cell mediated response to the SARS-CoV-2 S protein at Days 14 and 182 post-fourth dose of PHH-1V in Cohort 2 and to assess the safety and tolerability of PHH-1V as a fourth dose. Exploratory objectives included the assessment of reported COVID-19 severe infections occurring ≥14 days after booster and throughout the study. Additional analysis not included in the study protocol was performed: a descriptive cohort comparison of 4th dose with PHH-1V and due to clinical and public-health relevance of new emerging variants, a neutralizing antibody analysis against Omicron XBB.1.5 was performed.

Study vaccine

PHH-1V was supplied in vials, each containing 10 ready-to-use doses of 0.5 mL (40 µg). There was no requirement to dilute or reconstitute the vaccine. PHH-1V was shipped to clinical sites and kept refrigerated at 2–8°C. Vials were not allowed to be frozen.

Procedures and outcomes

All eligible participants to receive a fourth dose of PHH-1 V on Day 0 (open-label extension phase) were provided with a paper diary on Day 0 and returned to the site on Days 14. At day 0, 14, 98 and 182 (final visit) blood sample collection and safety follow-up were conducted.

Titres of neutralizing antibodies were determined by the inhibitory concentration 50 (IC_{50}, reported as reciprocal dilution) using a PBNA as described previously.[41] The GMT and the geometrical mean fold rise (GMFR) for adjusted treatment were calculated.

The T-cell-mediated immune response against the SARS-CoV-2 spike glycoprotein and spike protein RBD sequence were assessed after the *in vitro* peptide stimulation of peripheral blood mononuclear cells (PBMC) from vaccinated participants followed by enzyme-linked immune absorbent spot (ELISpot) and intracellular cytokine staining (ICS) with several cytokines on baseline and at Days 14 and 182 post-fourth dose of PHH-1 V in a subset of individuals from Cohort 2. Peptide pools of overlapping SARS-CoV-2 peptides, each encompassing the SARS-CoV-2 Protein S (two pools (Spike A and B) covering two different regions from spike S1 protein from Wuhan) or RBD domain (six peptides' pools covering RBD domain of spike protein from Wuhan-Hu-1, Beta, Delta variants and Omicron BA.1, BA.2 and XBB.1.5 subvariants) were used, details of procedures have been published previously[13] and a descriptive on Supplementary methods.

As suggested by the regulatory agency, the primary study outcome measure was neutralization antibody titer against Omicron BA.1, measured as the IC_{50} using PBNA and reported as $\log_{10}$ concentration for each individual sample and GMT, at Day 14 post-fourth dose with PHH-1 V in Cohort 2 from the extension HIPRA-HH-2 study versus post-

third dose with BNT162b2 in Cohort 2 from initial HIPRA-HH-2 study.

The secondary outcome measures were: neutralization titers against Beta and Delta variants, and Omicron BA.1, BA.4/5, XBB.1.5 subvariants measured as the IC_{50} using PBNA and reported as log_{10} concentration for each individual sample and GMT, at Days 14, 98 and 182 post-fourth dose of PHH-1 V versus baseline in both cohorts; the GMFR in neutralizing antibody titers for all studied variants at Days 14, 98 and 182 post-fourth dose of PHH-1 V versus baseline. T-cell-mediated immune response at baseline and Days 14 and 182 against SARS-CoV-2 Wuhan, Beta, Delta and Omicron variants.

Exploratory efficacy endpoint regarding severe COVID-19 infections occurrence from Day 14 post booster to Day 182 was assessed on the safety population.

Safety endpoints were solicited for local and systemic reactions from the time of vaccination until 7 days post-vaccination self-reported in the subject diary provided to participants at study start. Safety was also assessed by recording treatment-emergent AEs (TEAEs) with onset on or after the administration of study treatment through Day 28. Treatment-emergent was defined as any AE with onset on or after the administration of study treatment through Day 28 or any event that was present at baseline but worsened in intensity or was subsequently considered drug related by the Investigator through the end of the study. Serious AEs (SAEs), AEs of special interest (AESI) and medically attended AEs (MAAEs) were reported throughout the study. AEs were assessed at each visit based on careful clinical observation of the subject, laboratory tests or spontaneous reports by the subject discovered as a result of general questioning by the study staff. All AEs were recorded in the eCRF. All AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 26.0 coding system. The following was recorded for each event: indication, severity (grade 1, 2, 3 and 4),[42] duration (start and stop dates), seriousness, causal relationship with the intervention vaccine, actions taken, and outcome. The Investigator had to report any underlying condition when a surgical or medical procedure was required as the event term, and the procedure as an action taken. For a preexisting condition that had worsened in terms of severity or frequency, the meaning of the change had to be specified (e.g., worsening of hypertension). For all AEs, the Investigator had to pursue and obtain information adequate both to determine the outcome of the AE and to assess whether it meets criteria for classification as a serious AE requiring expedited notification. Adverse events should be followed until the event resolved or stabilized at a level acceptable to the Investigator.

Statistical analysis

A sample size of 100 subjects was calculated to provide 90% power to detect non-inferiority of the fourth dose to the third dose for the primary endpoint Omicron BA.1 in Cohort 2 considering a 5% significance level, a non-inferiority margin of 1.5 and assuming a pooled standard deviation of 0.53. No sample size calculations were conducted for comparisons involving Cohort 1.

In this study, non-inferiority was to be determined if the upper bound of a 95% confidence interval (CI) surrounding the geometric mean ratio (GMT ratio) of the mean paired log-difference between the third dose and the fourth dose responses was below 1.5. If the upper bound of the 95% CI was also below 1, superiority was concluded. Criteria in concordance with Food and Drug Administration (FD) Guidance for Industry on Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines[43] which state that non-inferiority for a new influenza vaccine product could be claimed if the upper bound of a two-sided 95% CI surrounding the ratio of GMT for the control to investigational product does not exceed 1.5.

To investigate the endpoints of neutralization titers against viral subvariants, measured as IC_{50} by PBNA and reported as log_{10} concentration for each individual sample and GMT, at baseline and Days 14, 98 and 182 post-fourth dose of PHH-1V in Cohort 2 versus post-third dose, mixed models for repeated measures (MMRM) were used. Similarly, the comparison between the fourth dose in Cohort 1 versus the third dose in Cohort 2 was assessed using MMRM models as well. In these models, the log_{10}-transformed neutralizing antibody measurements were used as response variable while the study visit, the dose and the dose-by-visit interaction terms were used as fixed effects. The age group factor was considered a covariate and the site, and the subject-nested-to-site were introduced as random effects. A compound symmetry covariance matrix structure was used. The denominator degrees of freedom were computed using the Kenward–Roger method. Weights were applied to the model estimation to account for sample distributions across covariates. The weighted LS mean estimates for each treatment dose were presented with the associated standard errors and 95% CIs for all visits. The back-transformed treatment group LS mean estimates and difference in weighted LS means (GMT ratio) were also presented for all visits with the corresponding 95% CI and p-value. GMFR analyses were conducted with the log_{10}-transformed post-baseline titer/baseline titer ratio using MMRM models as defined above.

Cellular immunogenicity analysis was analyzed providing two values for each parameter, imputing 0 in the event of negative values. ELISpot data were provided as Counts / 10^6 PBMCs. ICS data were provided as percentage therefore the data were divided by 100 for analysis. Also, MMRM were used for ELISpot data analysis and boxplot was used for graphical representation. For ICS data analysis, regression analysis was performed.

No formal hypothesis testing analysis of AE incidence rates was performed. Descriptive statistics were used for safety data reporting by cohorts and for the overall population.

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 the data; in writing of the

manuscript and in the decision to submit the manuscript for publication.

Results

In this open-label extension study, 301 subjects were screened of which 288 subjects were vaccinated with PHH-1V as a fourth dose and were distributed in two cohorts depending on the received previous vaccinations: Cohort 1 comprised a total of 106 subjects, all of them participants from previous HIPRA-HH-2 study, with two doses of BNT162b2 vaccine and one booster dose with PHH-1V; Cohort 2 included 182 subjects, 52 from the previous study and 130 subjects from the community (all having received two doses BNT162b2 vaccine and one additional dose BNT162b2 as booster) (Figure 1).

Subjects' demographics and baseline characteristics were balanced between cohorts and are shown in Table 1. For the overall population, mean age was 47.5 ± 14.86 years with 11.5% of the population aged 65 years or older. Baseline data from Cohort 2 community subjects reveal similar characteristics to those subjects already participating from the HIPRA-HH-2 study (data available in Table S1). Participant disposition showed that 11 (3.8%) subjects prematurely discontinued, 7 (6.6%) in Cohort 1 and 4 (2.2%) in Cohort 2. Reasons for early discontinuation were lost to follow-up (n = 7, 2.4%), withdrawal (n = 2, 0.7%), other (n = 1, 0.3%) and protocol deviation (n = 1, 0.3%). The mean study duration was 5.9 months (range: 1.0–6.5 months).

Immunogenicity of PHH-1V as heterologous booster

The extension study met its primary endpoint; a significant increase in neutralizing antibodies at Day 14 post-administration of PHH-1V was observed from 1739.02 (95% CI: 18.30; 43.64) for the third dose with homologous booster to 4049.01 (95% CI: 2795.39; 5864.84) for the fourth dose as heterologous booster with PHH-1 V (GMT ratio third dose vs fourth dose of 0.43 [95% CI: 0.28; 0.65]; p value < 0.0001) against SARS-CoV-2 Omicron BA.1 variant (Figure 2). Similar titers were found at Day 14 between extension study Cohorts 1 and 2 with titers of 3521.41 (95% CI: 382.17, 822.56) and 3912.01 (95% CI: 2707.46, 5652.49), respectively (Supplementary Table S3).

Immunogenicity of PHH-1V booster dose

Humoral immune response of PHH-1V booster (fourth dose) against Beta, Delta, Omicron BA.1, Omicron BA 4/5 and

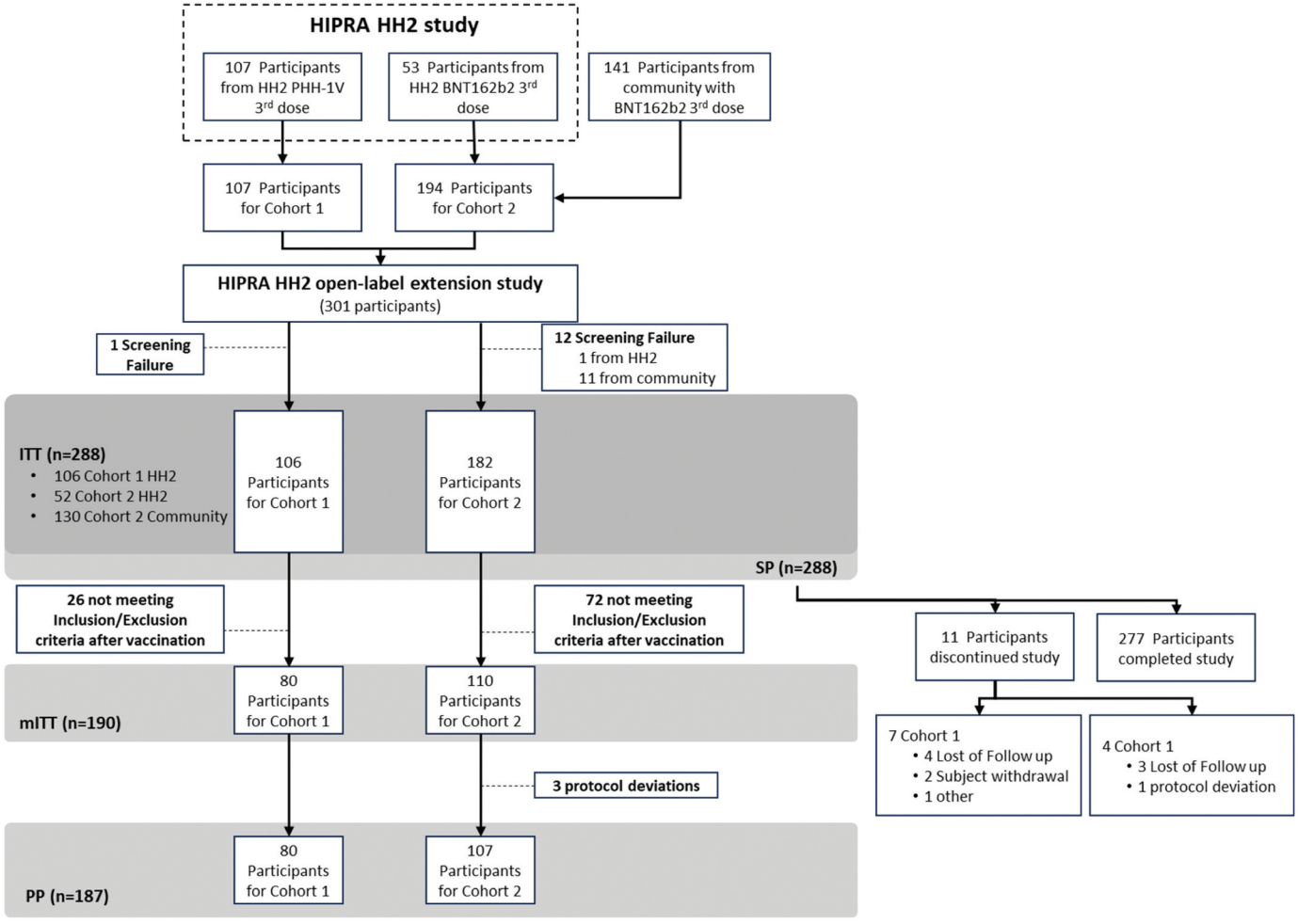

Figure 1. Participants disposition of HIPRA-HH-2 open-label extension study. Subjects who tested positive for COVID-19 within 14 days of receiving study drug were excluded.

Table 1. Subjects' demographics and baseline characteristics (safety population).

	Cohort 1: 4th dose PHH-1V (after[a] 2 doses of BNT162b2 + PHH 1V) (*n* = 106)	Cohort 2: 4th dose PHH-1V (after 3 doses of BNT162b2) (*n* = 182)	Overall (*n* = 288)
Mean age, years (SD)	49.0 (13.53)	46.6 (15.55)	47.5 (14.86)
≥65 years of age, n (%)	12 (11.3)	21 (11.5)	33 (11.5)
Sex, n (%)	42 (39.6)	73 (40.1)	115 (39.9)
Male	64 (60.4)	109 (59.9)	173 (60.1)
Female			
Race, n (%)	106 (100)	178 (97.8)	284 (98.6)
White	0	4 (2.2)	4 (1.4)
Other			
Median body mass index at screening[a], kg/m^2 (range)	24.34 (18.03–39.78)	24.92 (18.17–43.23)	24.59 (18.03–43.23)
Subjects with any medical history[b], n (%)	85 (80.2)	130 (71.4)	215 (74.7)
Hypertension	24 (22.6)	26 (14.3)	50 (17.4)
Menopause	14 (13.2)	24 (13.2)	38 (13.2)
Dyslipidaemia	9 (8.5)	9 (4.9)	18 (6.3)
Hypercholesterolaemia	4 (3.8)	13 (7.1)	17 (5.9)
Hypothyroidism	6 (5.7)	8 (4.4)	14 (4.9)
Post menopause	7 (6.6)	6 (3.3)	13 (4.5)
Asthma	4 (3.8)	7 (3.8)	11 (3.8)
Type 2 diabetes mellitus	2 (1.9)	8 (4.4)	10 (3.5)

[a]Body mass index was not available for 1 subject.
[b]Medical history events were coded using the MedDRA Dictionary, version 26.0, and only those with an incidence of >3% are included.
[c]n: Number of subjects.

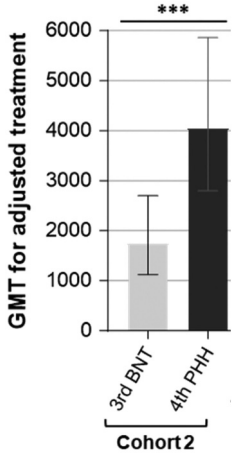

Figure 2. Comparison of neutralising antibody titre against SARS-CoV-2 Omicron BA.1 variant by PBNA. Representation of mean GMT for adjusted treatment and 95% CI at day 14 post-vaccination for third dose with BNT162b2 vaccine from HIPRA-HH-2 study (third BNT; *light grey column*) and fourth dose with PHH-1V from HIPRA-HH-2 extension study (fourth PHH; dark grey column). CI: confidence interval; GMT: Geometric mean titer; PBNA: pseudovirion-based neutralization assay. ***$p < .0001$.

Omicron XBB.1.5 variants were determined by neutralizing antibody GMTs and GMFRs for modified intention-to-treat (mITT) population (n = 190; Figure 3 and Table S2). Fourth dose with PHH-1V induced a statistically significant increase in neutralizing antibody titer on Day 14 for all variants compared with baseline titers. GMFRs (95% CI) at Day 14 were 6.96 (5.23, 9.25) for Beta variant, 6.27 (4.79, 8.22) for Delta variant, 9.21 (5.57, 15.21) for Omicron BA.1 variant, 11.80 (8.29, 16.80) for Omicron BA.4/5 variant and 5.22 (3.97, 6.87) for Omicron XBB.1.5 variant. Neutralizing antibody titer results over time (Figure 3 and Table S2) revealed a decline in neutralizing antibodies titers for all variants at 3 and 6 months after receiving the booster immunization but titers remained significantly higher compared with baseline levels for the overall mITT population.

Cohort analysis comparison of neutralizing antibody titers of the fourth dose with PHH-1V (Cohort 1: Homologous fourth dose, - *n* = 80; Cohort 2: Heterologous fourth dose, *n* = 110) were similar across subgroups for any variant at Day 14, 3 months and 6 months after the PHH-1 V booster (Table S3). Higher neutralizing antibodies were observed in cohort 1 compared to cohort 2 at baseline for all pre-specified variants with the exception of Omicron XBB.1.5 (Table S3).

SARS-CoV-2-specific T-cell responses after PHH-1V heterologous booster were evaluated in 15 participants from Cohort 2 by ELISpot and intracellular cytokine staining from PBMCs at baseline, and at Day 14 and 6 months after dose. *In vitro* re-stimulation of PBMCs from participants with SARS-CoV-2-derived peptide pools induced a significant IFN-γ T-cell response at Day 14 that persisted for at least 6 months (Figure 4). PHH-1V fourth dose significantly increased ($p < .0001$) the number of IFN-γ^+ spot forming cells (SFC) that responded to the *in vitro* PBMCs re-stimulation with RBD (Wuhan, Beta, Delta, Omicron BA.1, Omicron BA.2 and Omicron XBB.1.5) and Spike A peptides pools on Day 14 compared with baseline (Figure 4). At 6 months after the fourth dose, increases compared with baseline were still significant for the number of IFN-γ^+ SFC that responded to the RBD Omicron BA.1 and Omicron XBB.1.5 variants ($p < .05$), and trends ($0.05 < p < .1$) to higher values were observed in response to the stimulation with RBD Wuhan and Omicron BA.2. Results from ELISpot against Omicron XBB.1.5 variant demonstrated that a fourth dose of PHH-1V elicited a higher IFN-γ^+ T-cell response at Days 14 (78.70 IFN-γ^+ spots/10^6 PBMCs [range: 10.63–216.25]) and 182 after the booster (50.67 IFN-γ^+ spots/10^6 PBMCs [range: 3.75–135.00]) compared with baseline (19.64 IFN-γ^+ spots/10^6 PBMCs [range: 0.00–50.00]).

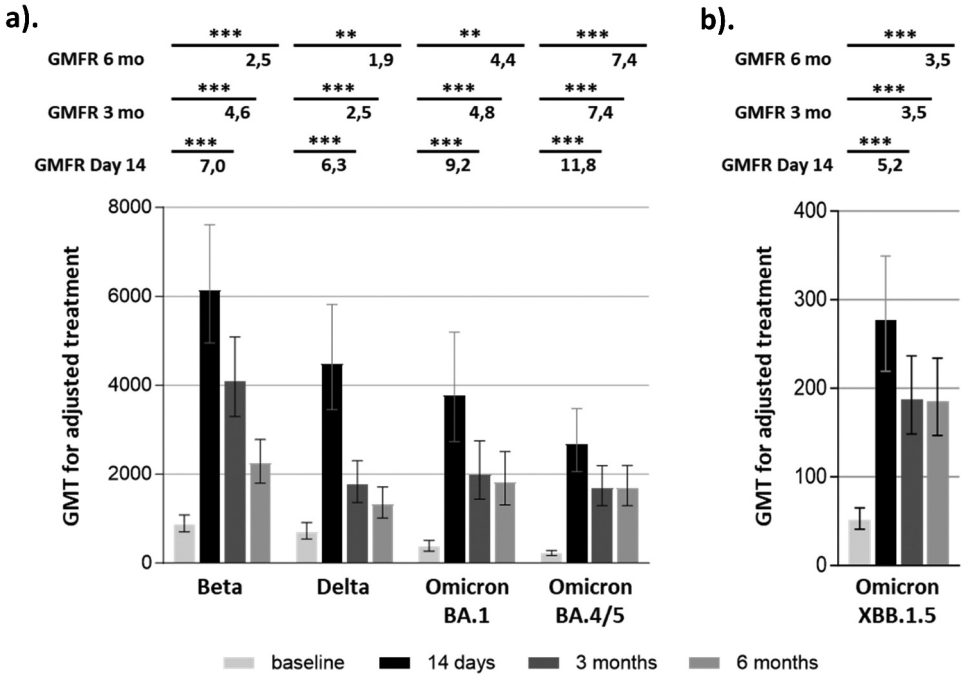

Figure 3. Neutralising antibody levels against SARS-CoV-2 variants by PBNA over time. Representation of mean GMT for adjusted treatment with 95% CI (columns) and mean GMFR from baseline (upper numbers) for all participants from mITT population treated with fourth dose of PHH-1V ($n = 190$) against SARS-CoV-2 Beta, Delta, Omicron BA.1, omicron BA.4/5 (a) and omicron XBB.1.5 variants (b) at baseline (*light grey*), day 14 (*black*), 3 months (*dark grey*) and 6 months (*grey*) post-dose. Subjects who reported COVID-19 infections were excluded from the reported day onwards. CI: confidence interval; GMFR: Geometric mean fold rise; GMT: Geometric mean titer; mITT: modified intention-to-treat population; PBNA: pseudovirion-based neutralization assay. ***$p < .0001$; **$p < .001$.

In the same subpopulation of 15 subjects from Cohort 2, the cellular immune response was analyzed by ICS on $CD4^+$ or $CD8^+$ T-cells (Figure 5). ICS results showed that the stimulation of PBMC with the RBD (Wuhan, Beta, Delta, Omicron BA.1 and Omicron BA.2 variants) and Spike A peptide pools significantly induced a higher activation of $CD4^+$ IFN-γ^+ T-cells at Day 14 compared with baseline. The percentage of $CD4^+$ IFN-γ^+ T-cells responding in vitro to the RBD and Spike A stimulus decreased on Day 182 and no significant differences were observed in any T-cell responses at this time point compared with baseline (Figure 5a). Although a trend to higher values of $CD8^+$ IFN-γ^+ T-cells at Day 14 compared with baseline were observed after stimulation with RBD (Beta, Delta, Omicron BA.1 and Omicron BA.2 variants) and Spike A peptide pools, no statistically significant differences were observed (Figure 5b). No clear induction was seen for IL-4 or IL-2 (data not shown).

Efficacy

At the end of the study, 36 subjects experienced non-severe COVID-19 infections (15 (14.2%) in Cohort 1 and 21 (11.5%) in Cohort 2). No subject experienced a severe COVID-19 infection, was hospitalized, admitted to the ICU, or died due to COVID-19 (Table S4).

Safety

A total of 859 TEAEs were reported in 246 (85.4%) subjects, including 307 TEAEs in 92 (86.8%) subjects in Cohort 1 and 552 TEAEs in 154 (84.6%) subjects in Cohort 2. No subjects experienced a serious TEAE or a TEAE leading to death. Overall, 759 TEAEs were reported as mild in intensity in 188 (65.3%) subjects, 91 TEAEs were reported as moderate in intensity in 51 (17.7%) subjects, and 9 TEAEs were reported as severe in intensity in 7 (2.4%) subjects. The most frequent AEs were injection site pain (Cohort 1: 84.0%; Cohort 2: 77.5%) and fatigue (Cohort 1: 17.9%; Cohort 2: 29.1%) (Table 2). COVID-19 was reported as a TEAE in 4 (3.8%) subjects from Cohort 1 and 2 (1.1%) subjects from Cohort 2.

One SAE was reported in 1 (0.3%) subject from Cohort 1 during the extension phase. This SAE of thermal burn was assessed as unrelated to the study drug by the Investigator and Sponsor.

Discussion

In the current endemic situation with emerging Omicron variants and waning protection, it is necessary to have booster vaccines with a broad response capability (breadth) to the different variants that also provide a long-lasting immune response. In addition, their inclusion in vaccination programs for persons at risk of suffering severe disease (such as those over 60 years old, those with underlying diseases or immunocompromised persons) is crucial. The usefulness of booster doses against SARS-CoV-2 to prevent long COVID-19 has also been recently demonstrated.[18,44] However, SARS-CoV-2 evolution, mainly driven by mutations in RBD allowing viral escape from neutralizing antibodies, is responsible for limited vaccine efficacy, as has been observed with the emergence of the Omicron variant despite two-dose vaccination of WH1.[45] Heterologous, rather than homologous, vaccination provides additional support for a mix-and-match approach[46] and may

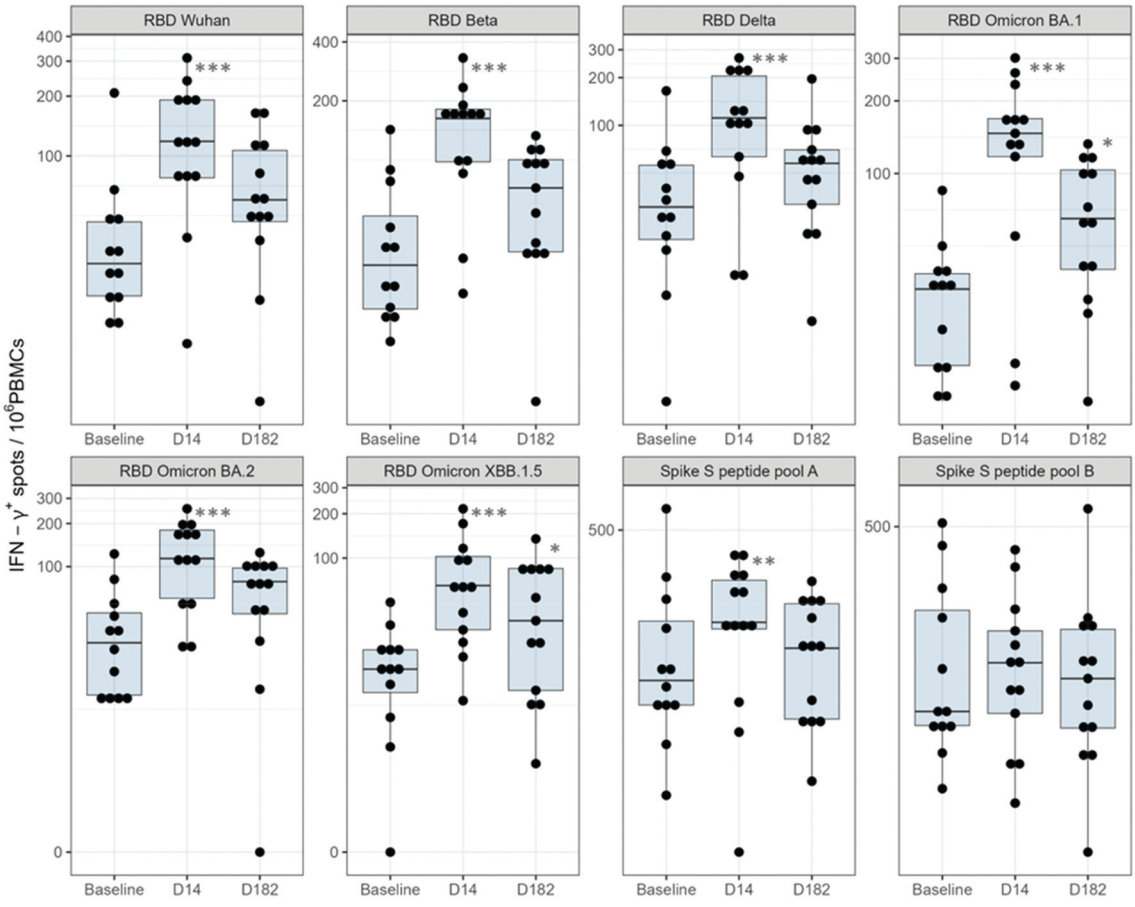

Figure 4. Total IFN-γ producing T cells upon PBMC re-stimulation with SARS-CoV-2 derived peptide pools by ELISpot. Frequencies of INF-γ^+ cells determined by ELISpot assay in re-stimulated PBMC from participants with PHH-1V heterologous booster (cohort 2; *n*=15) isolated before (baseline), 2 weeks (day 14) and 6 months (day 182) after fourth dose and re-stimulated with RBD Wuhan D614G, RBD 1.351 (beta), RBD B.1.617.2 (delta), RBD omicron BA.1, omicron BA.2, XBB1.5 and Spike (A and B) peptides pools. ***p< .0001, **p< .01, *p< .05.

provide more opportunities to accelerate the global vaccination campaigns. Currently available evidence comes from studies that include vaccines based on mRNA platforms and existing mRNA vaccines have not demonstrated sufficient immunity duration (up to 6 months), although new generation vaccines with self-amplifying mRNA appear to be a new option to improve the immunogenicity duration, only non-inferiority to BNT162b2 has been shown.[47] Recently, subtle differences in the mechanisms by which the different vaccine platforms (mRNA-, adenoviral vector- and recombinant protein-based) elicit immune responses have emerged[48]: the two mRNA vaccines approved to date showed efficacy after dose one by means of non-neutralizing antibodies and moderate Th1 responses while adenovirus vaccines elicited polyfunctional antibodies and potent T cell responses.[48]

The PHH-1V vaccine has proven its value as a booster in people with different primo-vaccination schedules (mRNA and/or adenovirus) as it is able to generate a potent, broad, and long-lasting immune response.[13,37,38] The reason for this might be the result of several factors, including its dimeric structure and the use of adjuvant. PHH-1V is a bivalent antigen that allows the spike RBD sequence of two different SARS-CoV-2 variants to be contained in a single heterodimeric molecule. This heterodimer structure allows the booster-induced immune response to focus on an important region of the virus involved in target cell binding. The RBD sequence is immunodominant and accounts for 90% of serum neutralizing activity.[28] Furthermore, the adjuvant enhances and induces an earlier, more robust and long-lasting immune response against the recombinant RBD heterodimer.[12,37,38] The fact that PHH-1V contains RBD sequences is very relevant since it is the main target of neutralizing antibodies (90% of the neutralizing activity is associated with this region).[49]

Results of our open-label extension study are consistent with previous findings[13,37]and demonstrate that PHH-1V is a robust immunogenic booster. The study met its primary objective and showed a significant increase in humoral immune response against the SARS-CoV-2 Omicron BA.1 variant using a heterologous booster with PHH-1V as a fourth dose versus a homologous booster as a third dose. The PHH-1V booster dose as fourth dose after a primary immunization either in participants with three doses of BNT162b2 or those who received two doses of BNT162b2 and one dose of PHH-1V elicited an immune response and a cross-reactivity among all subvariants tested. Although a decline on immune response over time is shown, neutralizing antibody titers were superior after a fourth dose of PHH-1 V compared with baseline on Days 14, 98 and 182 days irrespective of treatment cohort. Moreover, on the randomized, double-blind, controlled part before data presented herein,

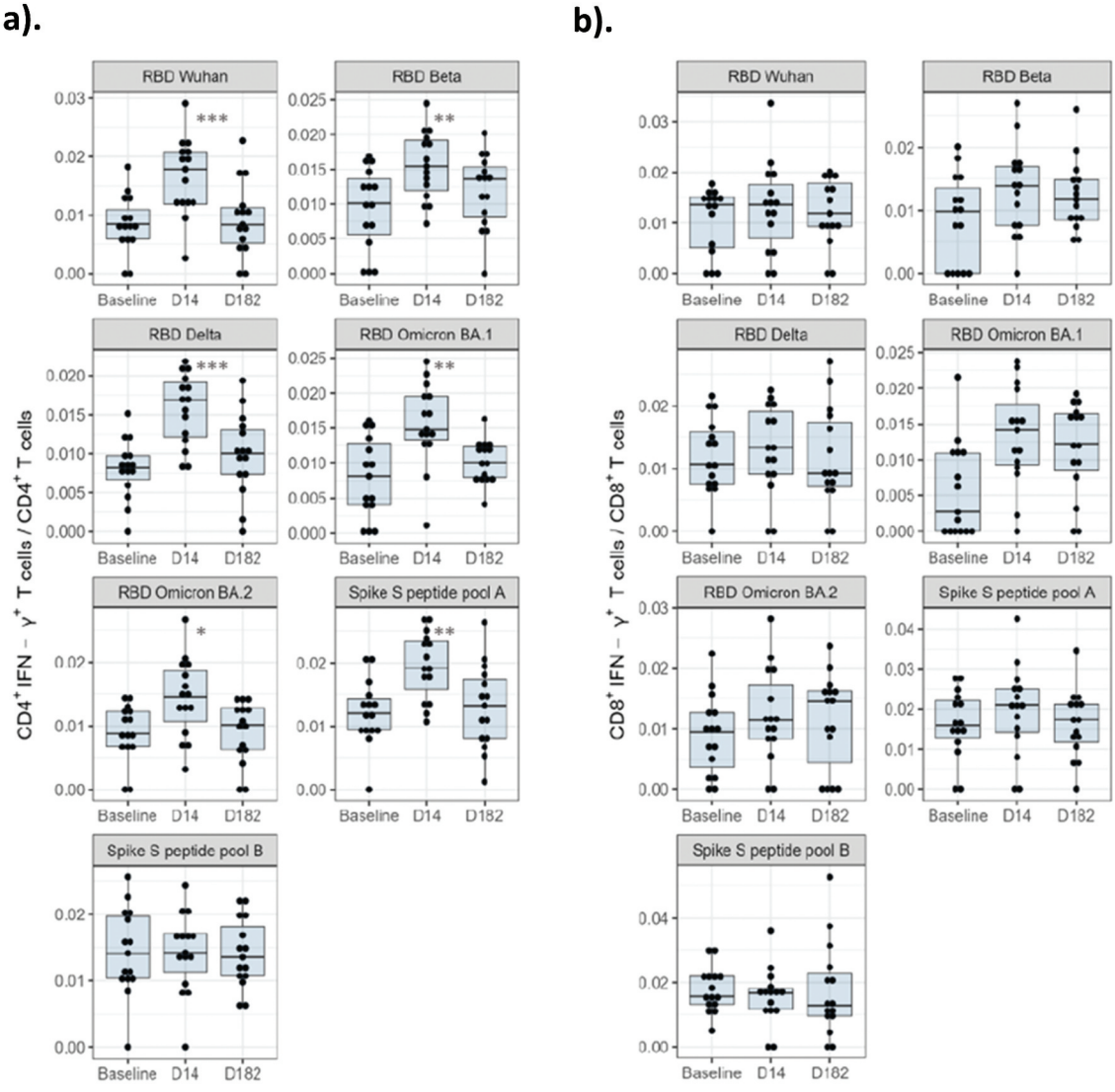

Figure 5. IFN-γ producing $CD4^+$ and $CD8^+$ T-cells upon PBMC re-stimulation with SARS-CoV-2-derived peptide pools by ICS. The frequencies of IFN-γ expressing $CD4^+$ T-cells (a) or $CD8^+$ T-cells (b) are shown. PBMC were isolated from PHH-1V heterologous booster participants (Cohort 2; *n*= 15) before the immunisation (baseline), two weeks (day 14) and 6 months (day 182) after the fourth dose with PHH-1V, stimulated with RBD Wuhan D614G, RBD 1.351 Beta), RBD B.1.617.2 (Delta), RBD Omicron BA.1, Omicron BA.2 and Spike (A and B) peptides pools, respectively. The cytokine expression in medium-stimulated PBMC was considered as the background value and subtracted from peptide-specific responses. ***$p < .0001$, **$p < .001$, *$p < .01$.

a decline on neutralizing antibodies was also described at 3 and 6 month,[13,37] but neutralizing antibodies titer at 6 months were significantly higher compared to mRNA comparator for all SARS-CoV-2 variants tested.[37] Secondary endpoints revealed that, despite the reduced number of participants analyzed, the booster with PHH-1 V induced a specific IFN-γ^+ T-cell response against RBD peptides of SARS-CoV-2 Omicron BA.1, BA.2 and XBB.1.5 subvariants, showing the cross-reactivity of the cellular immune response induced by the PHH-1V vaccine. Moreover, the ICS results suggest that the booster with PHH-1V induced a CD4 Th1-biased cell response against all SARS-CoV-2 RBD variants tested on Day 14. However, the cellular immunity induced by the PHH-1V booster was not detected using ICS 6 months after immunization, although T-cell responses were detected by ELISpot after in vitro re-stimulation with RBD peptides, which could be due to differences in sensitivity of the assays. The T-cell immune response generates memory T cells specific to the antigen that evoked the response, a key success factor for a vaccine.

These results are consistent with the observations of the HIPRA-HH-2 study and confirm the induction of strong humoral and cellular immunogenicity by PHH-1V, used both as a third dose[35] and here as a fourth dose.

Safety data on PHH-1V as a fourth dose were consistent with the previously reported data following a third dose of PHH-1V.[13,37,38] Only one serious AE was reported in 1 subject, which was assessed as unrelated to the study drug. There were no cases of severe COVID-19 infection in the open-label extension part of the study. These findings indicate that PHH-1V administered as a fourth dose provided protection against severe, life-threatening, and fatal forms of SARS-CoV-2 infection. Safety data also demonstrated the low reactogenicity of the vaccine, particularly the low incidence of fever. PHH-1V administered either as a fourth or a third dose demonstrated a good safety profile.[13,37]

Table 2. Frequency of TEAEs up to day 28 by treatment group among the safety population of HIPRA-HH-2 extension study.

	Cohort 1: 4th dose PHH-1 V (after 2 doses of BNT162b2 + PHH 1 V) (*n* = 106), n (%)	Cohort 2: 4th dose PHH-1 V (after[a] 3 doses of BNT162b2) (*n* = 182), n (%)	Overall (*n* = 288), n (%)
Any TEAE[1], n (%)	92 (86.8)	154 (84.6)	246 (85.4)
Serious TEAE, n (%)	0	0	0
TEAE leading to death, n (%)	0	0	0
TEAE intensity, No. of subjects (%)[2]			
Mild	71 (67.0)	117 (64.3)	188 (65.3)
Moderate	19 (17.9)	32 (17.6)	51 (17.7)
Severe	2 (1.9)	5 (2.7)	7 (2.4)
TEAE relationship to study treatment[2]			
Unrelated[3]	2 (1.9)	3 (1.6)	5 (1.7)
Related[4]	90 (84.9)	151 (83.0)	241 (83.7)
Most common TEAEs n (%)			
Injection site pain	89 (84.0)	141 (77.5)	230 (79.9)
Headache	22 (20.8)	50 (27.5)	72 (25.0)
Fatigue	19 (17.9)	53 (29.1)	72 (25.0)
Myalgia/malaise	29 (27.4)	47 (25.8)	83 (28.8)
Injection site induration	9 (8.5)	18 (9.9)	27 (9.4)
Injection site erythema	5 (4.7)	14 (7.7)	19 (6.6)

[a]TEAEs reported through Day 28 after vaccination, with a frequency ≥10% in either of the cohorts.
[b]If a subject experienced more than one TEAE, the subject is counted once at the most severe or most related event.
[c]Unrelated adverse events are those classified as not related and unlikely related. [4]Related adverse events are those classified as possibly, probably, and related. If a TEAE has a missing relationship, it is assumed to be related to the study treatment for analysis purposes.
[d]n: Number of subjects; TEAE: treatment-emergent adverse event.

An additional key benefit of adjuvant-based vaccines, such as PHH-1V, is that the inclusion of the adjuvant amplifies the immune response and positively impacts both the duration of immunity (humoral/cellular) and the breadth of the response. The value of adjuvanted protein vaccine boosting reported here has also been demonstrated elsewhere. For example, the monovalent beta-adjuvanted MVB.1.351 vaccine resulted in a higher neutralizing antibody response against the original strain, the Beta variant and the Delta and Omicron BA.1 variants than those observed with the mRNA vaccine BNT162b2 and the MVD614 formulation.[50] A two-dose regimen of the NVX-CoV2373 vaccine, which contains the full-length spike glycoprotein of the prototype strain plus Matrix-M adjuvant, administered to adults conferred 89.7% (95% CI 80.2 to 94.6) protection against SARS-CoV-2 infection and high efficacy against the B.1.1.7 variant.[48] A post-hoc analysis showed an efficacy against the B.1.1.7 (or alpha) variant of 86.3% (95% CI 71.3 to 93.5) and against the non-B.1.1.7 variant of 96.4% (95% CI 73.8 to 99.5).[48] From a practical perspective, protein-based vaccines offer convenient logistic features. These vaccines can be refrigerated as a ready-to-use formulation that does not require reconstitution prior to use, making distribution easier.[12] These positive practical aspects make the PHH-1V vaccine convenient to use for forthcoming vaccination campaigns. In contrast, storage and transport of mRNA vaccines require rigorous temperature control, making them almost impossible to use in some countries.[51] Additionally, it should be noted that immunocompromised patients and those on immunosuppressant therapies were excluded from mRNA vaccine trials because the neutralizing antibodies resulting from vaccination can elicit an immunological cascade that may further deteriorate the general health of these individuals and increase the risk of viral infection.[51]

The immune response shown by PHH-1V booster against Wuhan, Beta, Delta and Omicron variants (BA.1; BA.4/5) in this extension study was comparable with the response triggered by PHH-1V in a previous study, while the XBB.1.5 neutralizing antibody titers were lower, which was consistent with the results of other vaccine boosters and with the neutralizing antibodies raised by a natural infection. It should be noted that both the vaccine (PHH-1V) and natural infection elicited a more discrete humoral response against XBB.1.5 compared with the other subvariants.[52] It should also be pointed out that the evaluation of efficacy was extremely positive in terms of the incidence of COVID cases with a total of 36 cases (12.5%) being recorded, none of which were severe, during the 6-month study period when there was a high prevalence of BQ.1 and XBB.1.5 variants.[52] The response to XBB.1.5 seems to confer sufficient protection against severe disease.[52]

The PHH-1V vaccine delivered as a booster dose induced a potent and significant neutralizing antibody response against all studied Omicron variants up to XBB.1.5 via the same mechanism demonstrated previously for variants such as Wuhan, Beta and Delta.[51] This confirms the broad-spectrum response of PHH-1V against the different emerging variants of COVID-19, including the XBB.1.5 subvariant although this is not as strong. In addition, studies with long follow-up (6–12 months) demonstrated that PHH-1V provides durable immune responses.[13,37] This open-label extension study also demonstrated that PHH-1V was well tolerated and safe regardless of the primary vaccination received or previous SARS-CoV-2 infection. These findings suggest that PHH-1V could be an appropriate strategy for implementing upcoming heterologous vaccination campaigns. Although the study has some clear limitations especially considering actual SARS-CoV-2 endemics and the risk groups definition for SARS-CoV-2 vaccination on present recommendations. At the study initiation, the situation was still with a pandemic with global vaccination campaigns, therefore the included population were healthy individuals without real focus on risk groups as recommended nowadays like elderly, individuals with

immunocompromised conditions or with comorbidities. This is a clear limitation to extrapolate results presented herein with the population who is currently recommended to be vaccinated. Nevertheless, additional studies are ongoing on individuals with immunocompromised conditions and on elderly participants. Another limitation for its extrapolation to general population is the clear lack of racial and ethnic diversity being 98.6% of study participants defined as white. Although no inclusion limitation on race and ethnicity were defined in the protocol, we have an over representation of participants defined as white in line with historical tendency in Spain of race and ethnicity trial enrollment that do not reflect the actual composition of Spanish society and this is something to take into account for future clinical trials to increase our efforts to ensure a more representative disparity on races and ethnicities on our trial populations to improve this issue. This will allow to generalize the results for all racial and ethnic diversity.

As conclusion, the PHH-1V vaccine delivered as a booster dose induced a potent and significant neutralizing antibody response against all studied Omicron variants up to XBB.1.5 via the same mechanism demonstrated previously for variants such as Wuhan, Beta and Delta.[50] This confirms the broad-spectrum response of PHH-1V against the different emerging variants of COVID-19, including the XBB.1.5 subvariant. In addition, previous studies with long follow-up (6–12 months) demonstrated that PHH-1V provides durable immune responses.[13,22] This open-label extension study also demonstrated that PHH-1 V induces significatively higher neutralizing antibodies compared to baseline 6 months after booster and it was well tolerated and safe regardless of the primary vaccination received or previous SARS-CoV-2 infection, although further studies will need to validate these data on a diverse population. These findings, together with the advantages on logistics and accessibility compared to broadly used vaccines, suggest that PHH-1V could be an appropriate strategy for implementing upcoming heterologous vaccination campaigns.

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Disclosure statement

JR Arribas has received consulting fees and payment for participating in advisory boards from Gilead Sciences, MSD, GSK, Eli Lilly, Roche, Pfizer and Sobi; honoraria for lectures and support for meetings and/or travel from MSD.

P Munoz has speaker and/or consultant fees from BioMerieux, Gilead, Pfizer, Tillots, Mundipharma, Roche, Menarini and different scientific societies and nonprofit foundations of Fundación de Ciencias de la Salud, UIMP, Future day Foundation, Fundación Areces.

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Notes on contributor

Alex Soriano: I am Specialist in Internal Medicine and PhD in the University of Barcelona in 2006 and with 20 years working as Infectiologist in Hospital Clínic of Barcelona. My scientific contribution is focused on the treatment and management of bacteremia, infections in Intensive Care Units, and infections related to orthopedic implants. I have more than 400 articles in national and international journals with more than 16.000 citations and an H index of 65 (Google Scholar). I am the current Head of Infectious Diseases Department of Hospital Clínic of Barcelona and the Leader of the Nosocomial Infection Group of Institut d'Investigació en Biomedicina Agustí Pi-Sunyer (IDIBAPS) and the Past- President of European Bone and Joint Infection Society (EBJIS.org). Since 2015 co-Editor in chief of "*Infectious Diseases and Therapy*" and Co-author of "Mensa Anti-infective guide" pocketbook and application for iPhone and Android.

ORCID

María Jesús López http://orcid.org/0000-0001-5714-0125
Maria Del Mar Vazquez http://orcid.org/0000-0002-5571-0582
José Ramón Arribas http://orcid.org/0000-0002-7410-9450
Eunate Arana-Arri http://orcid.org/0000-0001-9759-333X
Patricia Muñoz http://orcid.org/0000-0001-5706-5583
José Molto http://orcid.org/0000-0003-4564-1963
Susana Otero-Romero http://orcid.org/0000-0002-6798-568X
Laia Bernad http://orcid.org/0000-0002-8137-640X
Raúl Pérez-Caballero http://orcid.org/0000-0001-7063-5958
Montserrat Plana http://orcid.org/0000-0002-0767-4329
Júlia G. Prado http://orcid.org/0000-0002-5439-4645
Álex Soriano http://orcid.org/0000-0002-9374-0811

Author contributions statement

All authors had full access to all study data, interpreted the data, provided critical conceptual input, critically reviewed and revised the manuscript and approved the decision to submit for publication. MJL, MMV, MA, JRA, EAA, PM, JNP, RR, JM, SOR and AS are PI from the participating

hospitals and listed in order of subject contribution. MP, IE, EA, RP, JGP, RPC, and LB were involved specifically in cellular immune response assay development, data generation and data analysis.

Data availability statement

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 of HIPRA S.A.

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