

Supplementary material

Title: Immunogenicity of Heterologous SARS-CoV-2 PHH-1V Protein Booster vaccination in People with Immunocompromising Conditions

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Eligibility criteria of the HIPRA-HH-4 trial

Inclusion criteria

Participants must meet all the following criteria to be considered eligible for the study:

1. Male, female, or transgender, ≥ 18 years old at Day 0.
2. Participant must provide consent indicating that she or he understands the purpose and potential risks and is willing and able to participate in the study and comply with all the study requirements and procedures (scheduled visits, laboratory tests, complete diaries, etc.).
3. Participant who has had one of the following Coronavirus Disease 2019 (COVID-19) vaccination schemes:

For sites in Spain, the following vaccination schemes were allowed:

- Three doses of mRNA vaccines (BNT162b2 [Comirnaty[®]; Pfizer-BioNTech] and/or mRNA-1273 [Spikevax[®]; Moderna]) with or without past history of positive test for SARS-CoV-2, provided that the last vaccination and diagnosis of SARS-CoV-2 are at least 91 days before Day 0, and if hospitalisation was required, the participant was discharged from hospital at least 30 days before Day 0.
- Two doses of mRNA vaccines (BNT162b2 and/or mRNA-1273) and a previous history of a positive test for SARS-CoV-2, provided that the last vaccination and diagnosis of COVID-19 are at least 91 days before Day 0, and if hospitalisation was required, the participant was discharged from hospital at least 30 days before Day 0.

For sites in Turkey where vaccination with CoronaVac (CoronaVac[®]; Sinovac) was started, the following vaccination schemes were allowed:

- A complete primary scheme of the following vaccines: CoronaVac and/or BNT162b2, with or without additional boosters of the same vaccines, with the last vaccination administered at least 91 days before Day 0. Participants with or without a history of a positive test for SARS-CoV-2 are allowed. The infection (first day of positive test, rapid antigen test [RAT], or PCR) must have been at least 91 days before Day 0.
4. Participant who has any of the following underlying immunosuppressive conditions:
 - Confirmed HIV infection with CD4⁺ T-cell counts < 400 cells/mm³ within the last 6 months prior to Day 0 regardless of plasma viral load (pVL) determination and antiretroviral (ARV) treatment;
 - Primary Antibody Deficiency Disorder on immunoglobulin replacement therapy for at least 6 months prior to Day 0 (maintenance dose);
 - Kidney disease on a dialysis programme for at least 6 months prior to Day 0;
 - Kidney transplant at least 1 year prior to Day 0, who has never been treated with anti-CD20/anti-CD3 or has received such treatment at least 1 year prior to Day 0, AND on maintenance immunosuppressive therapy based on at least 3 drugs: tacrolimus/cyclosporine, glucocorticoids and mycophenolate or everolimus/sirolimus.

- Auto-immune disease (including vasculitis, myositis, systemic lupus erythematosus/Sjögren, rheumatoid arthritis, multiple sclerosis, or other) on treatment with rituximab / ocrelizumab during at least 14 days within the last 6 months prior to Day 0.
5. Contraceptive use had to be consistent with local regulations for participants in clinical trials.
- a. Female participants of childbearing potential (defined as any female who has experienced menarche and until becoming postmenopausal* [defined as having ≥ 12 months of amenorrhea prior to screening without an alternative cause] unless she is surgically sterile):
- Have a negative pregnancy test on the day of vaccination.
 - Use any acceptable contraceptive method that should be started on Day 0 and until 8 weeks after vaccination, except hormonal contraception. Acceptable contraceptive methods are:
 - Hormonal contraception (progestogen-only or combined): oral, injectable, or transdermal (patch) started at least 28 days before Day 0 and until 8 weeks after vaccination.
 - Intrauterine device.
 - Vasectomised partner (the vasectomised partner should be the sole partner for that participant).
 - Sexual abstinence**, as a form of contraception, is acceptable if in line with the participant's lifestyle.
 - Condom.
- b. Male participants:
- Vasectomised participants.
 - Refrain from donating sperm for at least 28 days after Day 0.
 - Agree to use a male condom with partners who are women of childbearing potential from screening and for at least 8 weeks after vaccination.
 - Sexual abstinence**, as a form of contraception, is acceptable if in line with the participant's lifestyle.

**A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy.*

***Sexual abstinence is considered an effective method only if defined as refraining from heterosexual intercourse from screening until 8 weeks after receiving the vaccine for female participants and from screening until 4 weeks for male participants. Periodic abstinence (e.g., calendar, ovulation) and withdrawal are not acceptable methods of contraception.*

Exclusion criteria

Participants meeting any of the following criteria were excluded from the study:

1. History of anaphylaxis to any prior vaccine.
2. Participant received or plans to receive:
 - Live attenuated vaccines (licensed) within 4 weeks before or after receiving any study vaccine.
 - Other non-live vaccines (licensed) within 14 days before and after receiving any study vaccine.
 - Viral-vectored COVID-19 vaccines, such as Vaxzevria® (AstraZeneca) or Ad26.CoV2.S from Janssen.
3. Pregnancy or breastfeeding at screening or Day 0 (vaccination time-point) or willingness/intention to become pregnant during the entire length of the study for female participants. For male participants, willingness/intention that their partner becomes pregnant during the entire length of the study.

Medical conditions

4. A confirmed COVID-19 diagnosis (by RT-PCR or RAT, asymptomatic or symptomatic) < 90 days prior to Day 0.
5. Participant has a clinically significant acute illness (this does not include minor self-limited illness such as mild diarrhoea) or fever (temperature ≥ 38 °C [100.4 °F]) at screening or within 48 hours prior to the planned vaccination (Day 0).
6. Participant has had surgery requiring hospitalisation (defined as inpatient stay for > 24 hours) before vaccination and has not received hospital discharge by Day 0; or has surgery requiring hospitalisation planned within 12 weeks after study vaccine administration. Minor surgical procedures not requiring hospitalisation are accepted.
7. Participant has an ongoing severe and unstable psychiatric condition likely to affect participation in the study (*e.g.*, ongoing and non-stable severe depression, recent suicidal ideation, severe eating disorder, psychosis).
8. Participant has problematic or high-risk use of substances including alcohol (except tobacco) that can compromise the study follow-up. Problematic or risk use of psychoactive substances is understood as that which causes evident damage, whether it is dependence or any other physical, psychological, or social problem or that carries a high risk of suffering these damages. The negative consequences that consumption causes to third parties could be included.
9. Participant has a bleeding disorder (*e.g.*, factor deficiency, platelet disorder), blood dyscrasia, or continuous use of anticoagulants, or has any condition that in the opinion of the investigator contraindicates intramuscular injections or frequent phlebotomy. The use of ≤ 325 mg of aspirin or ≤ 75 mg of clopidogrel per day as prophylaxis is permitted but not combined.
10. Participant is suffering from post-acute COVID-19 syndrome / Long COVID.

Prior/Concomitant Therapy and Clinical Study Experience

11. Participant received any immunotherapy (monoclonal antibodies, plasma) aimed at preventing or treating COVID-19 within 90 days preceding the planned administration of the study vaccine (180 days in the case of Evusheld®)*.
12. Participation in any research involving an investigational product (drug, biologic, device) within 12 weeks prior to vaccination and during the study.
13. Participant has donated ≥ 450 ml of blood products within 12 weeks before screening.
14. Participant has any medical condition and/or finding that, in the investigator's opinion, might increase participant risks, interfere with the study or impair interpretation of study data.

**Treatment with immunotherapies (monoclonal antibodies, plasma) aimed at preventing COVID-19 (PrEP) is allowed at any time point of the study after Day 14 following current local guidelines. Those participants will be censored for the clinical endpoints on COVID-19 acquisition and disease outcomes, and the immune analysis will be controlled by the treatment received.*

Study populations

- Intent-to-treat (ITT): All participants who were enrolled, regardless of the individual's treatment status in the study. "Enrolled" was defined as answering "Yes" to the question "Has subject been recruited into the study?" in the eCRF.
- Modified Intent-to-treat (mITT): All individuals in the ITT who met the inclusion/exclusion criteria, received a dose of study vaccine and did not test positive for COVID-19 within 14 days after receiving the study vaccine. Participants in Spain were required to have a valid N-serology result at Screening or at Baseline.
- Immunogenicity population (IGP): All individuals in the mITT who had a valid immunogenicity test result, based on neutralisation titre, before receiving the study vaccine and at least one valid result after dosing. The participants were analysed according to their underlying disease, history of prior COVID-19 and/or positive N-serology and primary vaccination schemes (mRNA or CoronaVac/mRNA).
- Safety population (SP): All enrolled individuals who received the study vaccine. This population was used for all safety analyses. The participants were analysed according to their underlying disease and primary vaccination schemes (mRNA or a combination of an inactivated virus vaccine (CoronaVac) and mRNA vaccines).

Supplementary methods

Humoral immunogenicity assays

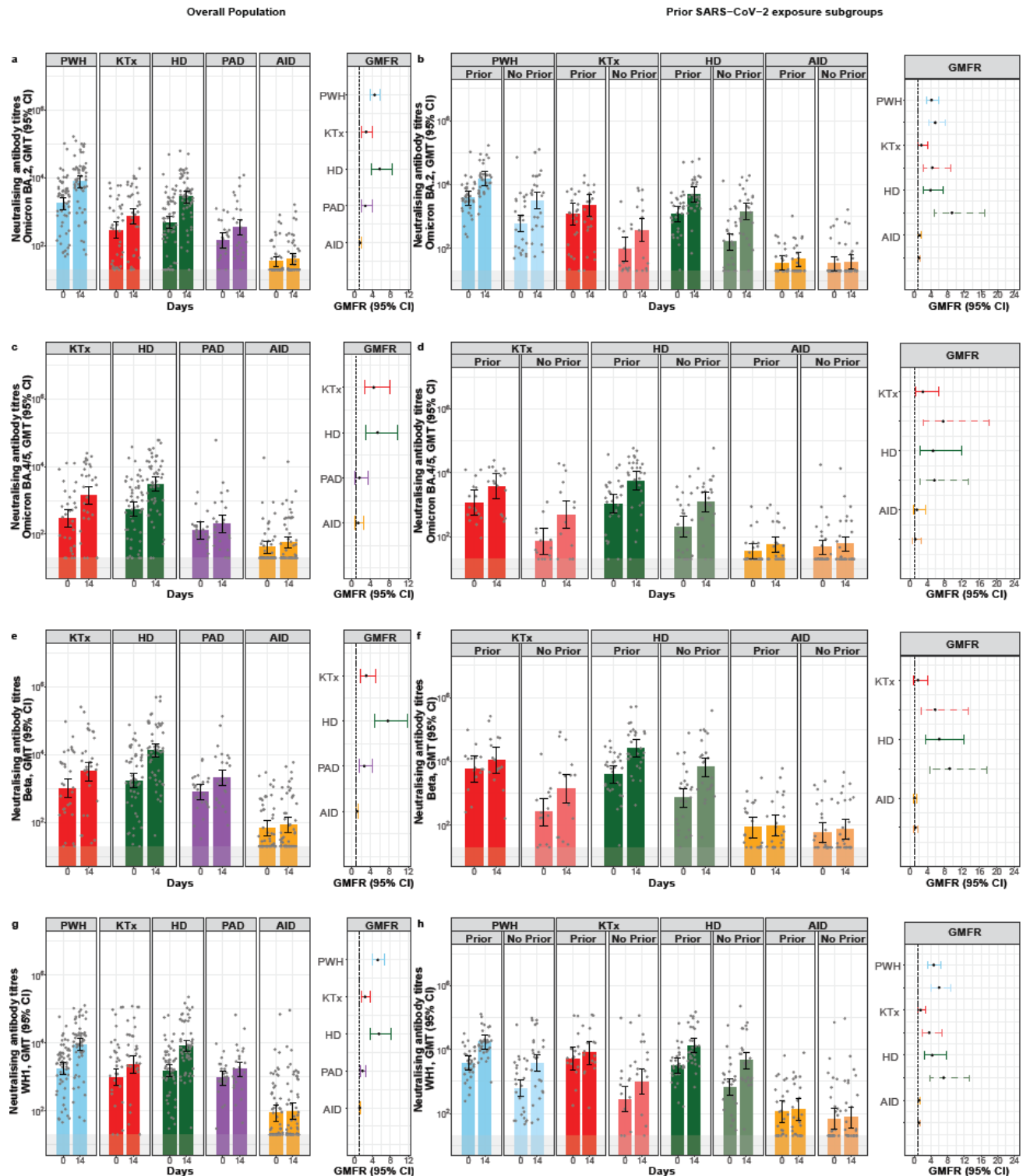
Neutralising antibody titres were measured as inhibitory dilution 50 (ID50) using a pseudovirus-based neutralisation assay (PBNA) with luciferase-reporter HIV-based lentiviral pseudoviruses bearing SARS-CoV-2 variant-specific spike proteins¹. Heat-inactivated sera were serially diluted from 1:20 and incubated with a previously titrated pseudovirus before infection of ACE2-overexpressing HEK293T cells. Luminescence was measured 48 h later using Britelite™ Plus luciferase substrate (PerkinElmer, Waltham, MA, USA) and an EnSight™ multimode reader (PerkinElmer). Neutralisation percentages were calculated relative to infected and uninfected controls, and ID50 values were obtained by four-parameter logistic regression in GraphPad Prism. In PWH, where PBNA was unsuitable due to the interference of antiretroviral drugs, a virus neutralisation assay (VNA) was used to assess neutralisation capacity employing replication-competent viruses and infecting Vero E6 cells² with Omicron BA.2, in substitution of Omicron BA.1 due to its reduced cytopathic effect in the assay³, and WH1 D614G viruses.

Cellular immunogenicity assays

Seven different pools of 15-mer peptides overlapping by 11 amino acids and spanning different regions of SARS-CoV-2 proteins were used to stimulate PBMCs. These pools included two that covered the whole spike (S1+S2) protein sequence and five that covered the receptor-binding domain (RBD) only, using sequences from the variants ancestral WH1, Beta (B.1.351), Delta (B.1.617.2), and Omicron BA.1 and BA.2. Descriptions of the peptide pools used are as follows:

- SPIKE_SA: 181 peptides covering the S1-2016 to S1-2196 region of the spike protein from the ancestral WH1 strain.
- SPIKE_SB: 181 peptides covering the S1-2197 to S2-2377 region of the spike protein from the ancestral WH1 strain.
- RBD_WH1: 84 peptides covering the RBD region from the WH1 sequence.
- RBD_Beta: 84 peptides covering the RBD region, including 12 mutations from the WH1 sequence.
- RBD_Delta: 84 peptides covering the RBD region, including 8 mutations from the WH1 sequence.
- RBD_Omicron BA.1: 84 peptides covering the RBD region, including 32 mutations from the WH1 sequence.
- RBD_Omicron BA.2: 84 peptides covering the RBD region, including 29 mutations from the WH1 sequence.

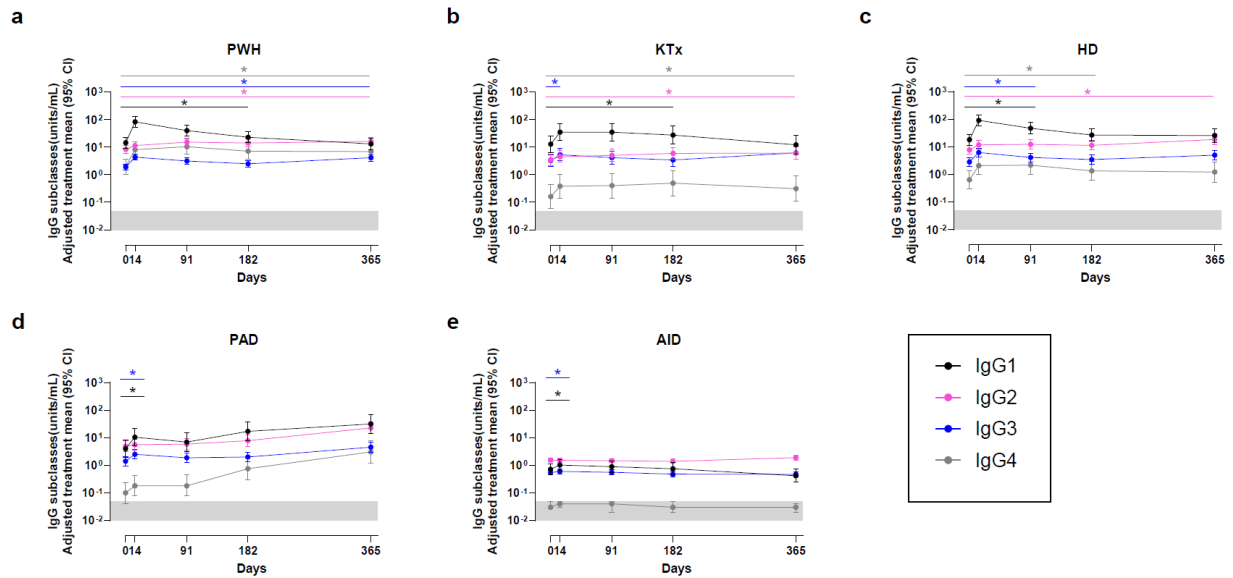
RBD-specific T-cells in the IFN- γ ELISpot assays were reported as the mean number of spot-forming cells per 10^6 PBMCs (spot-forming cells [SFCs]/ 10^6 PBMCs) across duplicates for each peptide pool, and as the percentage of cytokine-positive cells, after background subtraction in the intracellular cytokine staining (ICS) assay, provided that values were ≥ 0 after background subtraction. Negative values were set to 0.



Supplementary Figure S1 | Neutralising antibody responses at Day 0 and Day 14 against different SARS-CoV-2 variants.

Neutralising antibody titres measured by PBNA, or VNA for the PWH group, are shown (**a, c, e, g**) at Day 0 and Day 14 for each immunocompromising condition; and (**b, d, f, h**) stratified by prior SARS-CoV-2 exposure (solid lines, Prior-CoV-2; dashed lines, No Prior-CoV-2). Variants tested included (**a-b**) BA.2; (**c-d**) Omicron BA.4/5, the dominant circulating Omicron subvariants during the study period; (**e-f**) Beta, as the vaccine-matched variant; and (**g-h**) the ancestral WH1 strain. Individual determinations are shown as grey dots, and the bars represent the GMT (95% CI), shown as horizontal black lines, obtained by fitting the MMRM model using log-transformed data. The shaded grey area depicts the limit of detection of the assay (*i.e.*, $ID_{50} = 20$ reciprocal serum dilution). The right-hand box in each panel shows the GMFR (95% CI) as dots, from Day 0 to Day 14, calculated by ANOVA using log-transformed data. All analyses were performed in the mITT population ($N = 228$) with available data and in subgroups of > 10 participants.

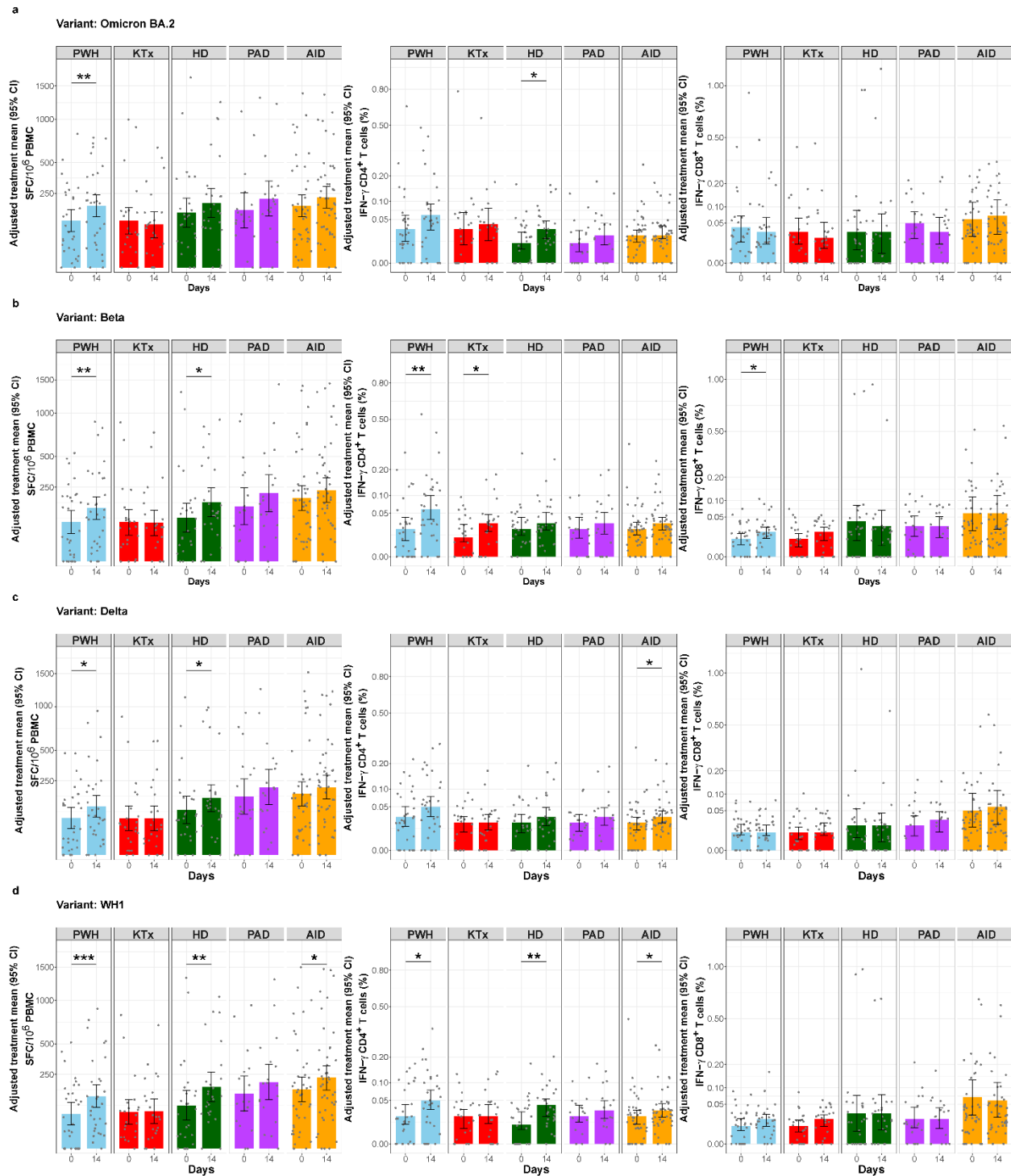
AID, participants with autoimmune disease receiving anti-CD20 therapy; ANOVA, analysis of variance; CI, confidence interval; GMFR, geometric mean fold rise; GMT, geometric mean titre; HD, participants on chronic haemodialysis/peritoneal dialysis; ID_{50} , inhibitory dilution 50; KTx, kidney transplant recipients; mITT, modified intention-to-treat; MMRM, mixed-effects model for repeated measures; No Prior, participants with no prior exposure to SARS-CoV-2; PAD, participants with primary antibody deficiencies; PBNA, pseudovirus-based neutralisation assay; Prior, participants with prior exposure to SARS-CoV-2; PWH, people with HIV; VNA, virus neutralisation assay.



Supplementary Figure S2 | Longitudinal IgG subclasses against SARS-CoV-2 Omicron BA.1 variant.

IgG subclass levels (IgG1, IgG2, IgG3, and IgG4; colour-coded) to SARS-CoV-2 RBD Omicron BA.1 are shown for each immunocompromising condition (a-e). All analyses were performed in the mITT population ($N = 228$) with available data and in subgroups of > 10 participants. Beyond Day 14, data were censored at the latest available timepoint prior to a confirmed new SARS-CoV-2 infection, administration of a new booster vaccination, and/or passive monoclonal antibody infusion (*i.e.*, Evusheld®). For participants with PAD, analyses of data beyond Day 14 were censored as participants received chronic replacement IgG therapy every 3 weeks. The shaded grey area depicts the limit of detection for the assay. $*P < 0.05$ is shown for the last time point at which significant differences were detected.

AID, participants with autoimmune disease receiving anti-CD20 therapy; *CI*, confidence interval; *HD*, participants on chronic haemodialysis/peritoneal dialysis; *KTx*, kidney transplant recipients; *mITT*, modified intention-to-treat; *PAD*, participants with primary antibody deficiency; *PWH*, people with HIV; *RBD*, receptor-binding domain.

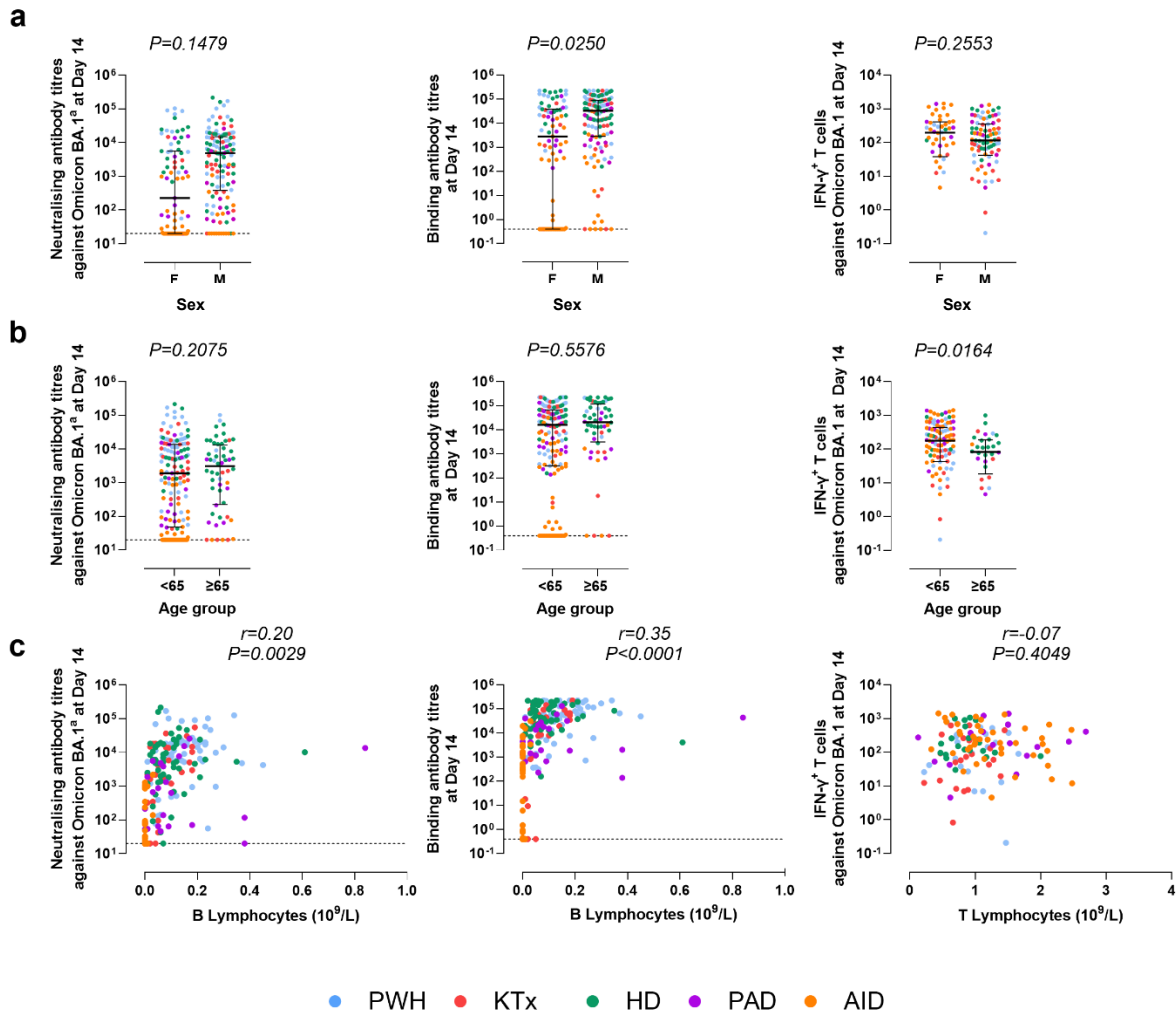


Supplementary Figure S3 | Cellular response at Day 0 and Day 14 against different SARS-CoV-2 variants.

(a) Magnitude of RBD-specific T-cell responses measured by IFN- γ ELISpot assay (left), and frequencies of RBD-specific IFN- γ CD4⁺ (centre) and IFN- γ CD8⁺ (right) T cells measured by ICS after restimulation with RBD Omicron BA.2; (b) Beta; (c) Delta; and (d) WH1 variant peptides at Day 0 and Day 14 for each

immunocompromising condition. Individual determinations are shown as grey dots. The bars represent the adjusted treatment mean and its 95% CI, indicated by horizontal black lines, obtained by fitting the MMRM model using square-root-transformed data. All analyses were performed in the IGP-T population (N = 141) with available data. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

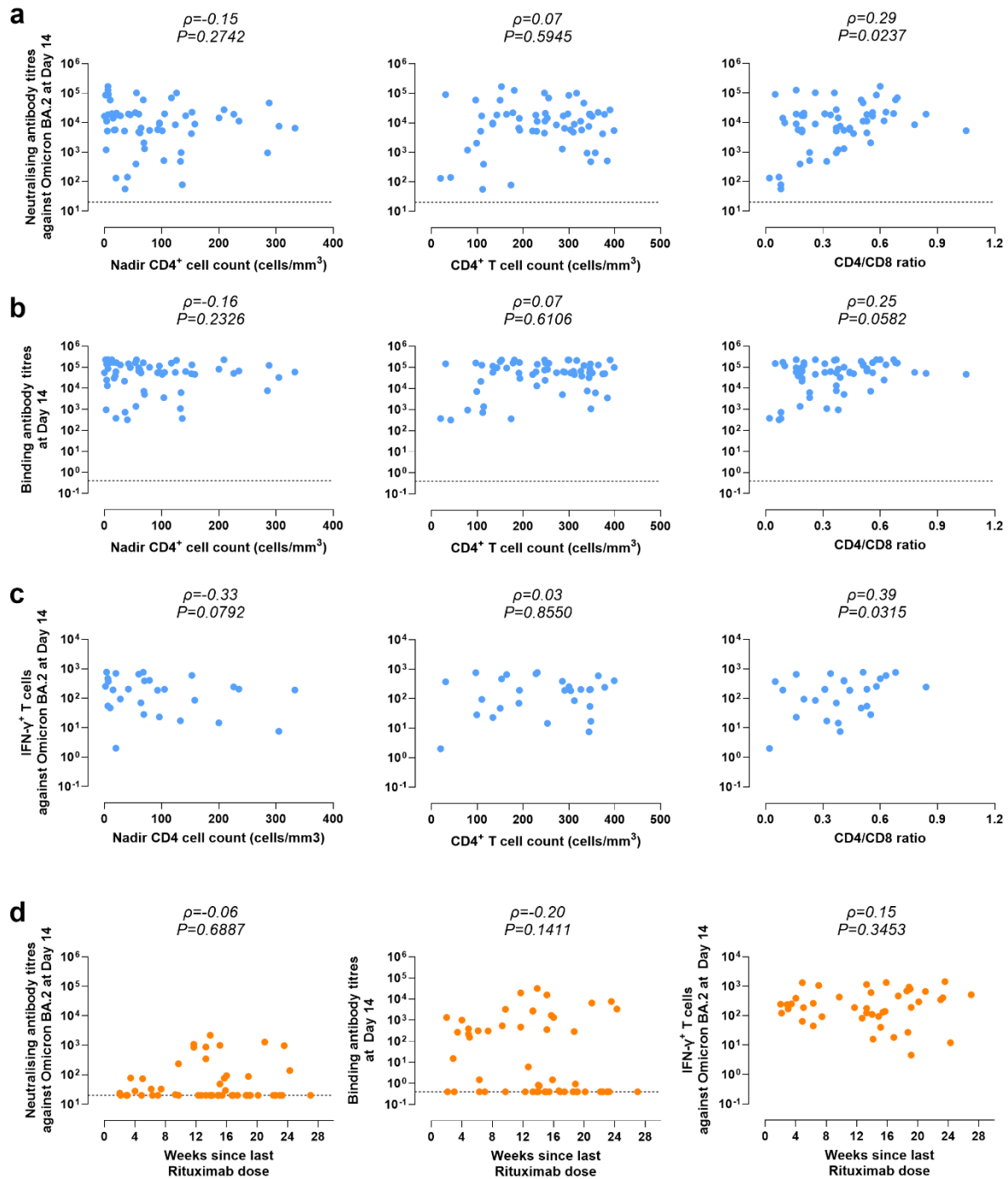
AID, participants with autoimmune disease receiving anti-CD20 therapy; CI, confidence interval; ELISpot, enzyme-linked immunosorbent spot; HD, participants on chronic haemodialysis/peritoneal dialysis; ICS, intracellular cytokine staining; IFN- γ , interferon γ ; IGP-T, T-cell immunogenicity population; KTx, kidney transplant recipients; MMRM, mixed-effects model for repeated measures; PAD, participants with primary antibody deficiency; PBMC, peripheral blood mononuclear cells; PWH, people with HIV; RBD, receptor-binding domain; SFC, spot-forming cell.



Supplementary Figure S4 | Demographic and clinical determinants of humoral and cellular post-vaccination immune responses.

(a) Comparisons of neutralising antibody titres measured by PBNA against Omicron BA.1 (^aVNA against BA.2 for PWH) (left), binding antibody levels (centre), and frequencies of RBD-specific IFN- γ ⁺ T cells against Omicron BA.1 (right) at Day 14, by sex assigned at birth; and (b) by age < 65 or ≥ 65 years. Horizontal black lines represent the median and IQR, and P-values from the Mann–Whitney test are indicated. (c) Pearson’s correlation of neutralising antibody titres against Omicron BA.1 by PBNA (^aVNA against Omicron BA.2 for PWH) at Day 14 with peripheral total B lymphocyte counts at Day 0 (left); binding antibody levels with peripheral total B lymphocyte count before vaccination (centre); and frequencies of RBD-specific IFN- γ ⁺ T cells against Omicron BA.1 at Day 14 with peripheral total T lymphocyte counts at Day 0 (right panel). Pearson’s correlation (r) and P-value (P) are indicated. Individual values are shown colour-coded for each immunocompromising condition. Limits of detection are shown as horizontal dashed lines (ID_{50} = 20 reciprocal serum dilution) for neutralising antibodies (left) and (0.40 U/mL) binding antibodies (centre).

AID, participants with autoimmune disease receiving anti-CD20 therapy; F, female; HD, participants on chronic haemodialysis/peritoneal dialysis; IFN- γ , interferon γ ; IQR, interquartile range; KTx, kidney transplant recipients; M, male; PAD, participants with primary antibody deficiency; PBMC, peripheral blood mononuclear cells; PBN, pseudovirus-based neutralisation assay; PWH, people with HIV; RBD, receptor-binding domain; SFC, spot-forming cells; VNA, virus neutralisation assay.

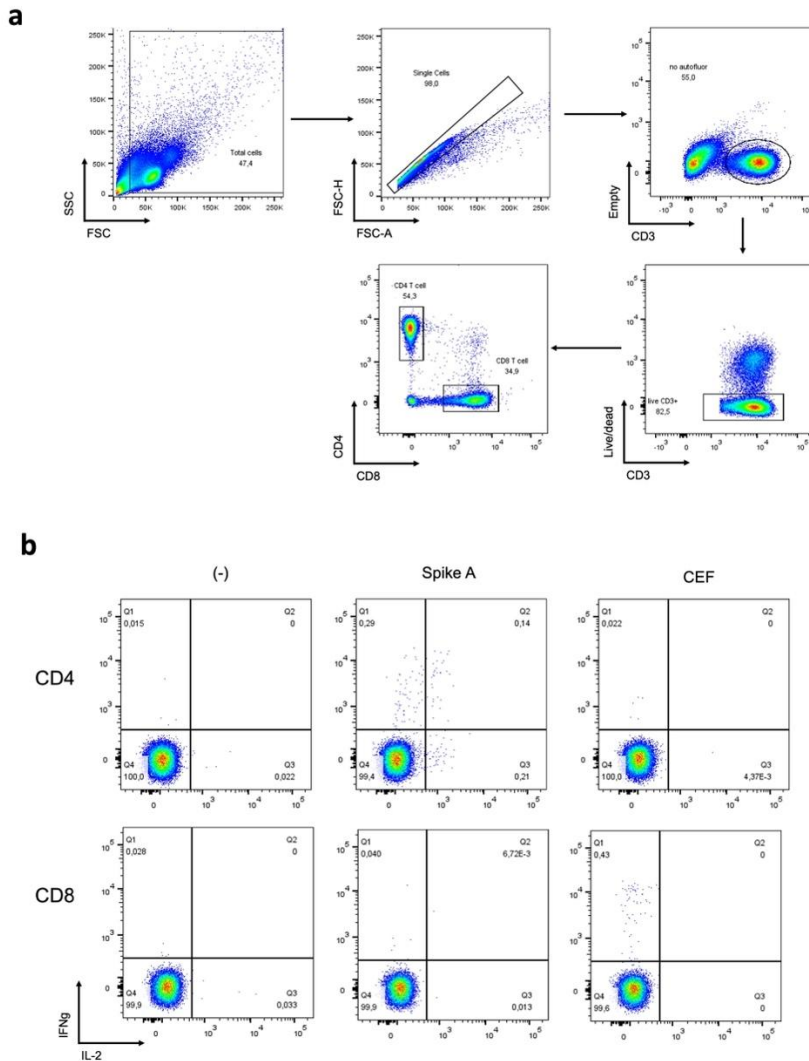


Supplementary Figure S5 | Clinical determinants of humoral and cellular post-vaccination immune responses in the PWH and AID groups.

(a) Spearman's correlation of neutralising antibody titres against BA.2 measured by VNA; (b) levels of binding antibodies; and (c) frequencies of RBD-specific IFN- γ ⁺ T cells measured by IFN- γ ELISpot assay against Omicron BA.2 at Day 14 with nadir CD4⁺ T-cell counts (left), absolute CD4⁺ T-cell counts (centre),

and the CD4/CD8 ratio before vaccination (right) in PWH. **(d)** Spearman's correlation of neutralising antibody titres against BA.1 by PBNA (left), levels of binding antibodies (centre), and frequencies of RBD-specific IFN- γ ⁺ T cells against Omicron BA.1 at Day 14 with the time elapsed between the last anti-CD20 administration and vaccination in the AID group. Spearman's rho (ρ) and P-value (P) are indicated. Individual values are shown in blue for the PWH group and orange for the AID group.

AID, participants with autoimmune disease receiving anti-CD20 therapy; ELISpot, enzyme-linked immunosorbent spot; IFN- γ , interferon γ ; PBMC, peripheral blood mononuclear cells; PBNA, pseudovirus-based neutralisation assay; PWH, people with HIV; RBD, receptor-binding domain; SFC, spot-forming cells; VNA, virus neutralisation assay.



Supplementary Figure S6 | Gating strategy of ICS in PBMCs.

(a) Example of the gating strategy used for ICS in PBMCs. First, total cells were selected by excluding cellular debris (SSC-A vs FSC-A). Then, single cells were separated from doublets (FSC-H vs FSC-A). Next, CD3-positive cells were selected, excluding autofluorescent and CD3-negative cells (Empty vs CD3-PerCP). Subsequently, CD3-positive live cells were determined (live/dead vs CD3-PerCP) and then CD4 T cells and CD8 T cells were selected (CD4-BV421 vs CD8-BV510). Finally, CD4 and CD8 T cells positive for IL-2, IFN- γ , and IL-2/IFN- γ were determined (IFN- γ -APC vs IL-2-PE); **(b)** Representative flow cytometry dot plots gated on CD4⁺ T cells and CD8⁺ T cells, in the absence of peptide stimulation (basal condition [-]), stimulation with spike A peptide pool (spike A), or CEF peptide pool (CEF) for IL-2 and IFN- γ expression.

APC, allophycocyanin; BV421, Brilliant Violet 421; BV510, Brilliant Violet 510; CEF, cytomegalovirus, Epstein-Barr virus and influenza virus peptide pool; FSC-A, forward scatter area; FSC-H, forward scatter height; ICS,

intracellular cytokine staining; PBMCs, peripheral blood mononuclear cells; PE, phycoerythrin; PerCP, peridinin chlorophyll protein; SSC-A, side scatter area.

Supplementary Table S1 | TEAEs up to 28 days after the PHH-1V booster administration for the overall ITT/SP, categorised by immunosuppressive condition group.

	PWH (N = 61)		KTx (N = 37)		HD (N = 59)		PAD (N = 24)		AID (N = 57)		Overall, ITT/SP (N = 238)	
	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)
TEAEs^a												
Total	123	40 (65.57)	86	26 (70.27)	74	31 (52.54)	93	20 (83.33)	204	48 (84.21)	580	165 (69.33)
Intensity^a												
Grade 1	112	32 (52.46)	80	20 (54.05)	58	24 (40.68)	61	10 (41.67)	159	32 (56.14)	470	118 (49.58)
Grade 2	7	5 (8.20)	5	5 (13.51)	6	6 (10.17)	23	6 (25.00)	33	12 (21.05)	74	34 (14.29)
Grade 3	0	0 (0)	1	1 (2.70)	1	1 (1.69)	5	2 (8.33)	8	4 (7.02)	15	8 (3.36)
Grade 4	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (4.17)	0	0 (0)	1	1 (0.42)
Missing	4	3 (4.92)	0	0 (0)	9	0 (0)	3	1 (4.17)	4	0 (0)	20	4 (1.68)
Relationship to study treatment^a												
Related	118	37 (60.66)	78	23 (62.16)	53	25 (42.37)	78	17 (70.83)	190	47 (82.46)	517	149 (62.61)
Unrelated	5	3 (4.92)	8	3 (8.11)	21	6 (10.17)	15	3 (12.50)	14	1 (1.75)	63	16 (6.72)
Solicited local reactions^a												
Total	51	33 (54.10)	38	20 (54.05)	32	19 (32.20)	35	15 (62.50)	86	43 (75.44)	242	130 (54.62)
Intensity^a												
Grade 1	49	31 (50.82)	37	19 (51.35)	32	19 (32.20)	27	10 (41.67)	73	32 (56.14)	218	111 (46.64)
Grade 2	2	2 (3.28)	1	1 (2.70)	0	0 (0)	6	3 (12.50)	9	7 (12.28)	18	13 (5.46)
Grade 3	0	0 (0)	0	0 (0)	0	0 (0)	2	2 (8.33)	4	4 (7.02)	6	6 (2.52)
Grade 4	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)
Relationship to study treatment^a												
Related	51	33 (54.10)	38	20 (54.05)	32	19 (32.20)	35	15 (62.50)	86	43 (75.44)	242	130 (54.62)
Unrelated	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)
Solicited systemic events^a												
Total	65	21 (34.43)	37	12 (32.43)	32	16 (27.12)	47	12 (50.0)	94	29 (50.88)	275	90 (37.82)
Intensity												
Grade 1	61	17 (27.87)	37	12 (32.43)	23	14 (23.73)	30	7 (29.17)	70	21 (36.84)	221	71 (29.83)
Grade 2	4	4 (6.56)	0	0 (0)	2	2 (3.39)	14	4 (16.67)	20	7 (12.28)	40	17 (7.14)
Grade 3	0	0 (0)	0	0 (0)	0	0 (0)	3	1 (4.17)	4	1 (1.75)	7	2 (0.84)
Grade 4	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)
Missing	0	0 (0)	0	0 (0)	7	0 (0)	0	0 (0)	0	0 (0)	7	0 (0)

	PWH (N = 61)		KTx (N = 37)		HD (N = 59)		PAD (N = 24)		AID (N = 57)		Overall, ITT/SP (N = 238)	
	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)
Relationship to study treatment^a												
Related	65	21 (34.43)	36	12 (32.43)	21	15 (25.42)	42	11 (45.83)	91	27 (47.37)	255	86 (36.13)
Unrelated	0	0 (0)	1	0 (0)	11	1 (1.69)	5	1 (4.17)	3	2 (3.51)	20	4 (1.68)
Unsolicited adverse event^a												
Total	7	6 (9.84)	11	8 (21.62)	10	8 (13.56)	11	9 (37.50)	24	15 (26.32)	63	46 (19.33)
Intensity												
Grade 1	2	1 (1.64)	6	3 (8.11)	3	3 (5.08)	4	3 (12.50)	16	10 (17.54)	31	20 (8.40)
Grade 2	1	1 (1.64)	4	4 (10.81)	4	4 (6.78)	3	2 (8.33)	4	2 (3.51)	16	13 (5.46)
Grade 3	0	0 (0)	1	1 (2.70)	1	1 (1.69)	0	0 (0)	0	0 (0)	2	2 (0.84)
Grade 4	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (4.17)	0	0 (0)	1	1 (0.42)
Missing	4	4 (6.56)	0	0 (0)	2	0 (0)	3	3 (12.50)	4	3 (5.26)	13	10 (4.20)
Relationship to study treatment^a												
Related	2	1 (1.64)	4	3 (8.11)	0	0 (0)	1	1 (4.17)	13	6 (10.53)	20	11 (4.62)
Unrelated	5	5 (8.20)	7	5 (13.51)	10	8 (13.56)	10	8 (33.33)	11	9 (15.79)	43	35 (14.71)
Serious TEAEs^a												
Total	0	0	1	1 (2.70)	1	1 (1.69)	1	1 (4.17)	0	0 (0)	3	3 (1.26)
Intensity^a												
Grade 1	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)
Grade 2	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)
Grade 3	0	0 (0)	1	1 (2.70)	1	1 (1.69)	0	0 (0)	0	0 (0)	2	2 (0.84)
Grade 4	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (4.17)	0	0 (0)	1	1 (0.42)
Missing	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)
Relationship to study treatment^a												
Related	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)
Unrelated	0	0 (0)	1	1 (2.70)	1	1 (1.69)	1	1 (4.17)	0	0 (0)	3	3 (1.26)

^aIf a subject experienced more than one adverse event, the subject is counted once, based on the most severe or most related event.

TEAE: Adverse event that started on or after the date of administration of study treatment until 28 days thereafter. Related adverse events are classified as possibly, probably, or related. If a TEAE has a missing relationship, it is assumed to be related to the study treatment for analysis purposes. Unrelated adverse events are those classified as not related and unlikely related.

AID, participants with autoimmune disease receiving anti-CD20 therapy; HD, participants on chronic haemodialysis/peritoneal dialysis; ITT, intention-to-treat; KTx, kidney transplant recipients; PAD, participants with primary antibody deficiency; PWH, people living with HIV; SP, safety population; TEAE, treatment-emergent adverse events.

Supplementary Table S2 | Summary of serious adverse events and medically attended adverse events by MedDRA System Organ Class and Preferred Term for the overall ITT/Safety population, categorised by immunosuppressive condition group.

System Organ Class and Preferred Term	PWH (N = 61)		KTx (N = 37)		HD (N = 59)		PAD (N = 24)		AID (N = 57)		Overall (N = 238)	
	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)
Total number of SAEs	4	4 (6.56)	19	9 (24.32)	44	29 (49.15)	6	6 (25)	9	9 (15.79)	82	57 (23.95)
Infections and Infestations	1	1 (1.64)	9	4 (10.81)	15	13 (22.03)	4	4 (16.67)	3	3 (5.26)	32	25 (10.50)
Pneumonia	1	1 (1.64)	1	1 (2.70)	2	2 (3.39)	0	0 (0)	1	1 (1.75)	5	5 (2.1)
COVID-19	0	0 (0)	1	1 (2.70)	1	1 (1.69)	0	0 (0)	1	1 (1.75)	3	3 (1.26)
Sepsis	0	0 (0)	1	1 (2.70)	2	2 (3.39)	0	0 (0)	0	0 (0)	3	3 (1.26)
Bronchitis	0	0 (0)	0	0 (0)	1	1 (1.69)	1	1 (4.17)	0	0 (0)	2	2 (0.84)
COVID-19 pneumonia	0	0 (0)	0	0 (0)	2	2 (3.39)	0	0 (0)	0	0 (0)	2	2 (0.84)
Cellulitis	0	0 (0)	2	1 (2.70)	1	1 (1.69)	0	0 (0)	0	0 (0)	3	2 (0.84)
Klebsiella bacteraemia	0	0 (0)	1	1 (2.70)	1	1 (1.69)	0	0 (0)	0	0 (0)	2	2 (0.84)
Septic shock	0	0 (0)	1	1 (2.70)	1	1 (1.69)	0	0 (0)	0	0 (0)	2	2 (0.84)
Abscess	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (4.17)	0	0 (0)	1	1 (0.42)
Appendicitis perforated	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Bacteraemia	0	0 (0)	1	1 (2.70)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)
Bronchitis viral	0	0 (0)	1	1 (2.70)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)
Device related infection	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (4.17)	0	0 (0)	1	1 (0.42)
Diverticulitis	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Influenza	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (1.75)	1	1 (0.42)
Pneumonia bacterial	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Urinary tract infection	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (4.17)	0	0 (0)	1	1 (0.42)
Wound cellulitis	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Cardiac Disorders	0	0 (0)	4	3 (8.11)	4	4 (6.78)	1	1 (4.17)	1	1 (1.75)	10	9 (3.78)
Acute myocardial infarction	0	0 (0)	0	0 (0)	1	1 (1.69)	1	1 (4.17)	1	1 (1.75)	3	3 (1.26)
Cardiac failure congestive	0	0 (0)	2	1 (2.70)	1	1 (1.69)	0	0 (0)	0	0 (0)	3	2 (0.84)
Cardiac disorder	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)

System Organ Class and Preferred Term	PWH (N = 61)		KTx (N = 37)		HD (N = 59)		PAD (N = 24)		AID (N = 57)		Overall (N = 238)	
	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)
Cardiac failure	0	0 (0)	1	1 (2.70)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)
Cardiogenic shock	0	0 (0)	1	1 (2.70)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)
Coronary artery disease	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Neoplasms Benign, Malignant and Unspecified (incl Cysts And Polyps)	1	1 (1.64)	1	1 (2.70)	4	3 (5.08)	1	1 (4.17)	1	1 (1.75)	8	7 (2.94)
Adenocarcinoma of colon	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (4.17)	0	0 (0)	1	1 (0.42)
Anal squamous cell carcinoma	1	1 (1.64)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)
Bladder adenocarcinoma stage unspecified	0	0 (0)	1	1 (2.70)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)
Metastases to bone	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Neoplasm recurrence	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (1.75)	1	1 (0.42)
Parathyroid tumour benign	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Prostate cancer	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Squamous cell carcinoma of skin	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Gastrointestinal Disorders	0	0 (0)	1	1 (2.70)	6	5 (8.47)	0	0 (0)	0	0 (0)	7	6 (2.52)
Gastrointestinal haemorrhage	0	0 (0)	0	0 (0)	3	3 (5.08)	0	0 (0)	0	0 (0)	3	3 (1.26)
Diarrhoea	0	0 (0)	1	1 (2.70)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)
Gastric ulcer	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Incarcerated umbilical hernia	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Rectal haemorrhage	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Nervous System Disorders	1	1 (1.64)	0	0 (0)	2	2 (3.39)	0	0 (0)	1	1 (1.75)	4	4 (1.68)
Leukoencephalopathy	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Multiple sclerosis relapse	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (1.75)	1	1 (0.42)
Parkinson's disease	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Vertebrobasilar stroke	1	1 (1.64)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)
Vascular Disorders	0	0 (0)	1	1 (2.70)	3	2 (3.4)	0	0 (0)	1	1 (1.75)	5	4 (1.68)
Aortic stenosis	0	0 (0)	1	1 (2.70)	1	1 (1.69)	0	0 (0)	0	0 (0)	2	2 (0.84)

System Organ Class and Preferred Term	PWH (N = 61)		KTx (N = 37)		HD (N = 59)		PAD (N = 24)		AID (N = 57)		Overall (N = 238)	
	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)
Hypertension	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Peripheral ischaemia	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Vasculitis	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (1.75)	1	1 (0.42)
Injury, Poisoning and Procedural Complications	0	0 (0)	0	0 (0)	4	3 (5.08)	0	0 (0)	0	0 (0)	4	3 (1.26)
Graft thrombosis	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Hip fracture	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Post procedural urine leak	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Subdural haematoma	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Surgical and Medical procedures	0	0 (0)	0	0 (0)	3	3 (5.08)	0	0 (0)	0	0 (0)	3	3 (1.26)
Renal transplant	0	0 (0)	0	0 (0)	3	3 (5.08)	0	0 (0)	0	0 (0)	3	3 (1.26)
Hepatobiliary Disorders	0	0 (0)	1	1 (2.70)	0	0 (0)	0	0 (0)	1	1 (1.75)	2	2 (0.84)
Bile duct stone	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (1.75)	1	1 (0.42)
Cholangitis	0	0 (0)	1	1 (2.70)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)
Blood and Lymphatic System disorders	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Cytopenia	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Congenital, Familial and Genetic disorders	0	0 (0)	1	1 (2.70)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)
Congenital cystic kidney disease	0	0 (0)	1	1 (2.70)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)
General Disorders and Administration Site Conditions	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Pyrexia	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Metabolism and Nutrition Disorders	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (1.75)	1	1 (0.42)
Cachexia	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (1.75)	1	1 (0.42)
Psychiatric Disorders	1	1 (1.64)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)
Substance abuse	1	1 (1.64)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)
Renal and Urinary Disorders	0	0 (0)	1	1 (2.70)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)

System Organ Class and Preferred Term	PWH (N = 61)		KTx (N = 37)		HD (N = 59)		PAD (N = 24)		AID (N = 57)		Overall (N = 238)	
	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)
Renal impairment	0	0 (0)	1	1 (2.70)	0	0 (0)	0	0 (0)	0	0 (0)	1	1 (0.42)
Respiratory, Thoracic and Mediastinal disorders	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Chronic obstructive pulmonary disease	0	0 (0)	0	0 (0)	1	1 (1.69)	0	0 (0)	0	0 (0)	1	1 (0.42)
Total number of MAAEs	20	12 (19.67)	16	11 (29.73)	32	21 (35.59)	29	16 (66.67)	25	15 (26.32)	122	75 (31.51)
Related Grade 1	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)
Related Grade 2	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)
Related Grade 3	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)
Related Grade 4	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)
Unrelated Grade 1	5	4 (6.56)	7	6 (16.22)	13	9 (15.25)	11	8 (33.33)	20	12 (21.05)	56	39 (16.4)
Unrelated Grade 2	13	9 (14.75)	9	6 (16.22)	16	11 (18.64)	16	10 (41.67)	3	3 (5.26)	57	39 (16.4)
Unrelated Grade 3	2	1 (1.64)	0	0 (0)	2	2 (3.39)	0	0 (0)	0	0 (0)	4	3 (1.26)
Unrelated Grade 4	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)
Missing	0	0 (0)	0	0 (0)	1	1 (1.69)	2	2 (8.33)	2	2 (3.51)	5	5 (2.10)

If a participant experienced more than one SAE, the participant is counted once for each SOC and once for each PT.

SOCs are ordered in decreasing frequency of the total number of participants with SAEs reported in each SOC and PTs are ordered within a SOC in decreasing frequency of the total number of participants with each SAE.

Pooled related adverse events are those classified as possibly, probably and related. Pooled unrelated adverse events are those classified as not related and unlikely related.

Adverse events were coded using the MedDRA Dictionary, version 26.1.

AID, participants with autoimmune disease receiving anti-CD20 therapy; COVID-19, Coronavirus Disease 2019; HD, participants on chronic haemodialysis/peritoneal dialysis; ITT, intention-to-treat; KTx, kidney transplant recipients; PAD, participants with primary antibody deficiency; PWH, people living with HIV; TEAE, treatment-emergent adverse events; MAAEs, Medically Attended Adverse Events; MedDRA, Medical Dictionary for Regulatory Activities; PT, Preferred Term; SAE, serious adverse event; SOC, System Organ class.

Supplementary Table S3 | Summary of SARS-CoV-2 infections (ITT, Safety Population).

	PWH (N = 61)	KTx (N = 37)	HD (N = 59)	PAD (N = 24)	AID (N = 57)
Participants with SARS-CoV-2 infections $\geq$ 14 days after PHH-1V administration according to COVID-19 infection criteria throughout the study duration, n (%).	6 (9.84)	9 (24.32)	20 (33.90)	7 (29.17)	19 (33.33)
95% CI ^a	3.70, 20.19	11.77, 41.20	22.08, 47.39	12.62, 51.09	21.40, 47.06
Participants with COVID-19 severe infections $\geq$ 14 days after PHH-1V administration and through the end of the study requiring hospitalisation, n (%).	0 (0)	1 (2.70)	3 (5.08)	0 (0)	1 (1.75)
95% CI ^a	NA	0.07, 14.16	1.06, 14.15	NA	0.04, 9.39
Participants with ICU admissions associated with COVID-19 $\geq$ 14 days after PHH-1V administration and through the end of the study, n (%).	0 (0)	1 (2.70)	0 (0)	0 (0)	0 (0)
95% CI ^a	NA	0.07, 14.16	NA	NA	NA
Participants with non-invasive ventilation administration associated with COVID-19 $\geq$ 14 days after PHH-1V administration and through the end of the study, n (%).	0 (0)	1 (2.70)	3 (5.08)	0 (0)	1 (1.75)
95% CI ^a	NA	0.07, 14.16	1.06, 14.15	NA	0.04, 9.39
Participants with deaths associated with COVID-19 $\geq$ 14 days after PHH-1V administration and through the end of the study, n (%).	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
95% CI ^a	NA	NA	NA	NA	NA

^aA responder is defined as a participant who meets the criteria. Exact CI for the proportion of responders has been calculated using the Clopper-Pearson method.

AID, participants with autoimmune disease receiving anti-CD20 therapy; CI, confidence interval; COVID-19, Coronavirus Disease 2019; HD, participants on chronic haemodialysis/peritoneal dialysis; ITT, intention-to-treat; NA, not applicable; NC, nucleocapsid; KTx, kidney transplant recipients; PAD, participants with primary antibody deficiency; PWH, people living with HIV.

Supplementary Table S4 | Panel of flow cytometry antibodies used in the intracellular cytokine staining (ICS).

Antibody (clone)	Catalogue number	Source
CD28-Purified (L293)	348040	BD Pharmingen™, BD Biosciences, San Jose, CA, USA
CD49d-Purified (9F10)	304340	BioLegend, Inc., San Diego, CA, USA
CD3-PerCP (SIK7)	345766	BD Biosciences
CD4-BV421 (RPA-T4)	562424	BD Biosciences
CD8-BV510 (SK1)	563919	BD Biosciences
IFN- γ -APC (B27)	506510	BioLegend
IL-2-PE (5344.111)	340450	BD Biosciences

References (Supplementary Material)

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- 3 Shuai H, Chan JF-W, Hu B, *et al.* Attenuated replication and pathogenicity of SARS-CoV-2 B.1.1.529 Omicron. *Nature* 2022; **603**: 693–9.