

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

Beatriz Mothe

bmothe@irsicaixa.es

IrsiCaixa, Hospital Universitari Germans Trias i Pujol <https://orcid.org/0000-0001-9975-407X>

Susana Benet

Fight AIDS Foundation

Benjamin Trinité

IrsiCaixa <https://orcid.org/0000-0003-3809-042X>

Raúl Pérez-Caballero

IrsiCaixa

Antoni Castro

Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta (IDIBGI)

Dalia Raich Regue

<https://orcid.org/0000-0001-7656-5700>

Erola Ainsua-Enrich

IrsiCaixa

Ignasi Esteban

Fundació de Recerca Clínic Barcelona - Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)

Yovaninna Alarcón-Soto

Fundació Lluita contra les Infeccions

Edwards Pradenas

IRSI CAIXA <https://orcid.org/0000-0001-5162-8920>

Laia Bernad

IrsiCaixa

Sofia Sabato

Fight Against Infections, Infectious Diseases Department, Hospital Germans Trias i Pujol

Abelardo Hurtado Ganoza

Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta (IDIBGI) <https://orcid.org/0000-0002-4248-1418>

Roc Pumarol

Fundació de Recerca Clínic Barcelona - Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)

Nuria Pedreño-Lopez

AIDS Research Institute <https://orcid.org/0000-0001-6927-2301>

Alex Soriano

Hospital Clínic de Barcelona

Mete Tan

MetPharm Arastirma Gelistirme Saglik Danismanlik

Lucía Bailón

Department of Infectious Diseases, Hospital Universitari Germans Trias i Pujol, Badalona, Spain.

<https://orcid.org/0000-0001-7277-9314>

Andrea Vazquez

Fundació Lluita contra les Infeccions, Badalona, Spain.

Cristina Peligero-Cruz

Irsicaixa

Cristina Martínez Merchan

Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta (IDIBGI)

Estel Aparicio

IrsiCaixa

Rytis Boreika

IrsiCaixa, Germans Trias i Pujol Research Institute (IGTP)

Ruth Poderos

IrsiCaixa AIDS Research Institute

Elena Aurrecoechea

Fundació de Recerca Clínic Barcelona - Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)

Maria Luisa Rodriguez de la Concepción

IRSICAIXA <https://orcid.org/0000-0002-6944-3462>

Giorgio Barbareschi

European AIDS Treatment Group (EATG)

Christian Brander

IRSICAIXA AIDS Research Institute and Institució Catalana de Recerca i Estudis Avançats (ICREA)

<https://orcid.org/0000-0002-0548-5778>

Jose Molto

Fundació Lluita contra les Infeccions, Infectious Diseases Department, Hospital Germans Trias i Pujol

<https://orcid.org/0000-0003-4564-1963>

Montserrat Plana

Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)

Jorge Carrillo

IrsiCaixa <https://orcid.org/0000-0003-0221-5948>

Nuria Izquierdo-Useros

IrsiCaixa / Germans Trias i Pujol Research Institute (IGTP) / CIBER Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III <https://orcid.org/0000-0002-1039-1821>

Julià Blanco

IrsiCaixa <https://orcid.org/0000-0002-2225-0217>

Julia Prado

IrsiCaixa AIDS Research Institute <https://orcid.org/0000-0002-5439-4645>

Article

Keywords: HIPRA COVID-19 vaccine; Antibodies, Neutralising; T-Lymphocytes; Immunoglobulin G; Immunity; Primary Immunodeficiency Diseases; HIV Infections; Renal Dialysis

Posted Date: October 22nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11358870/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: **Yes** there is potential Competing Interest. C.B. was a shareholder and co-founder of AELIX Therapeutics and reports consultancy fees from Gilead Sciences, Astrivax and Omniscope. J.B. reports consultancy fees from NESAPOR EUROPE and HIPRA and, outside the presented work, institutional grants from MSD, NESAPOR EUROPE, and HIPRA. N.I.U. has collaborated with the PharmaMar Foundation as part of a scientific jury and reports institutional funding from Pharma Mar, HIPRA, Amassence, and Smart Vitamins. B.M. reports consultancy, advisory and/or speaker fees from AELIX Therapeutics, Gilead Sciences, Janssen, ViiV, and MSD. J.M. has received research funding, consultancy fees, and lecture sponsorships from, and has served on advisory boards for, MSD, Gilead Sciences, ViiV Healthcare, and Johnson & Johnson. The remaining authors declare that the research was conducted in the absence of any conflict of interest.

1 Word count: 199 (abstract); 6,527 (main text)

2 Display items: 7 (main figures); 2 (main tables)

3 References: 39 (main text)

4 **Article**

5

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

8

9 **Authors**

10 Susana Benet^{1,2*}, Benjamin Trinité^{3*}, Raúl Pérez-Caballero^{3,4*}, Antoni Castro^{5*}, Dàlia Raich-
11 Regué^{3*}, Erola Ainsua^{3*}, Ignasi Esteban^{6*}, Yovaninna Alarcón-Soto², Edwards Pradenas^{3,§}, Laia
12 Bernad³, Sofia Sabato^{1,2,7}, Abelardo Hurtado Ganoza⁵, Roc Pumarol⁶, Nuria Pedreño³, Alex
13 Soriano^{6,8}, Mete Tan⁹, Lucia Bailón^{1,2}, Andrea Vázquez², Cristina Peligero³, Cristina Martínez
14 Merchan⁵, Estel Aparicio³, Rytis Boreika³, Ruth Peña³, Elena Aurrecoechea⁶, María Luisa
15 Rodríguez de la Concepción³, Giorgio Barbareschi¹⁰, Christian Brander^{3,8,11,12}, Jose Moltó^{1,2,8},
16 Montserrat Plana^{6^}, Jorge Carrillo^{3,8^}, Nuria Izquierdo-Useros^{3,8^}, Julià Blanco^{3,4,8,11^}, Julia G.
17 Prado^{3,4,8^@}, Beatriz Mothe^{1,2,3,4,8,11^@}, and the RBDCOV HIPRA-HH-4 study group[†]

18

19 **Affiliations**

20 ¹Department of Infectious Diseases, Hospital Universitari Germans Trias i Pujol, Badalona, Spain

21 ²Fundació Lluita contra les Infeccions, Badalona, Spain

22 ³IrsiCaixa, Badalona, Spain

23 ⁴Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain

24 ⁵Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta (IDIBGI), Girona, Spain

25 ⁶Fundació de Recerca Clínic Barcelona - Institut d'Investigacions Biomèdiques August Pi i
26 Sunyer (IDIBAPS), Barcelona, Spain

27 ⁷Department of Medicine, Autonomous University of Barcelona, Cerdanyola del Vallès, Spain

28 ⁸Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC),
29 Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

30 ⁹MetPharm Arastirma Gelistirme Saglik Danismanlik, Istanbul, Turkiye
31 ¹⁰European AIDS Treatment Group (EATG), Brussels, Belgium
32 ¹¹Department of Infectious Diseases and Immunity, University of Vic-Central University of
33 Catalonia, Vic, Spain
34 ¹²Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain
35 [§]Current address: Facultad de Ciencias, Universidad San Sebastián, Concepción, Chile
36 ^{*}Shared first coauthors of this manuscript
37 [^]Shared last coauthors of this manuscript
38 [†]Members of the RBDCOV group are listed at the end of the Article
39 [@]corresponding authors
40

41 **Corresponding authors**

42 Julia G. Prado
43 IrsiCaixa, Germans Trias i Pujol Research Institute (IGTP), Ctra del Canyet s/n, 08916, Badalona,
44 Spain
45 (+34) 93 465 63 74
46 jgarciaprado@irsicaixa.es
47 Beatriz Mothe
48 Department of Infectious Diseases and Fundació Lluita contra les Infeccions, Hospital Germans
49 Trias i Pujol, Ctra del Canyet s/n, 08916, Badalona, Spain
50 (+34) 93 465 78 97
51 bmothe@lluïta.org
52

Abstract

Individuals with immunocompromising conditions face an increased risk of severe COVID-19 and often respond poorly to vaccination. HIPRA-HH-4 was a phase IIb/III, single-arm trial evaluating immunogenicity and safety of a PHH-1V booster, a protein-based adjuvanted SARS-CoV-2 vaccine, in 238 immunocompromised adults after mRNA SARS-CoV-2 vaccination. Recruited May-July 2022, participants included people living with HIV and < 400 CD4⁺ T cells (PWH, $n = 61$), post-kidney transplantation ($n = 37$), chronic haemodialysis (HD, $n = 59$), primary antibody deficiency (PAD, $n = 24$), and anti-CD20-treated autoimmune disease (AID, $n = 57$). By Day 14, PHH-1V increased binding and neutralising antibodies against Omicron BA.1, BA.2, and BA.4/5 in all groups except AID. Cross-reactivity was observed against SARS-CoV, but not other human coronaviruses. IgG1 and IgG3 titres increased across groups, whereas IgG4 and IgG2 responses varied. IgG avidity increased by Day 14 and remained elevated over 1 year. RBD-specific IFN- γ -secreting CD4⁺ T-cell responses occurred mainly in PWH and HD. Antibody and T-cell responses waned by Day 365, with more durable immunity in PWH and HD. PHH-1V was well tolerated, causing mostly mild or moderate, transient adverse events, and enhanced immune responses after one booster dose, informing future vaccine strategies for vulnerable groups.

Trial registration number: ClinicalTrials.gov ID: [NCT05303402](https://clinicaltrials.gov/ct2/show/study/NCT05303402), registered 31 March 2022.

Keywords

HIPRA COVID-19 vaccine; Antibodies, Neutralising; T-Lymphocytes; Immunoglobulin G; Immunity; Primary Immunodeficiency Diseases; HIV Infections; Renal Dialysis.

Introduction

People with immunocompromising conditions are at increased risk of severe outcomes from infectious diseases, including COVID-19.¹⁻⁴ This vulnerability can arise from both the underlying conditions and/or their immunosuppressive treatments, which impair innate and adaptive immunity. Consequently, vaccine-induced protective immunity in this population is often suboptimal, characterised by reduced and short-lived immune responses and limited prevention of SARS-CoV-2 infection acquisition and/or progression to severe COVID-19 disease. This leads to higher hospitalisation rates, poorer disease outcomes, and prolonged viral shedding,⁵ which has been linked to the emergence of new escape variants.^{5,6} The COVID-19 pandemic underscored the need to optimise antiviral treatments and tailor immunisation strategies for these at-risk groups. Booster vaccination has emerged as a critical tool to enhance and sustain immune protection^{7,8}. However, data on booster efficacy, cross-reactivity against evolving variants, the durability of post-booster responses over extended periods, and vaccine safety in immunocompromised individuals remain limited, partly due to their frequent exclusion and/or underrepresentation in clinical trials.^{7,9}

Recombinant protein-based adjuvanted vaccines, such as PHH-1V (BIMERVAX®, HIPRA HUMAN HEALTH, S.L.U., Spain), offer a promising alternative to mRNA-based boosters. Approved as a booster in March 2023,¹⁰ the first-generation PHH-1V vaccine was an adjuvanted, recombinant protein-based vaccine composed of a SARS-CoV-2 spike protein receptor-binding domain (RBD) fusion heterodimer from the Alpha (B.1.1.7) and Beta (B.1.351) lineages. The immunogen has demonstrated the ability to elicit strong immune responses against emerging variants, particularly Omicron^{11,12}, while minimising reactogenicity.^{11,13,14} PHH-1V has also been shown to enhance T-cell responses without Th2 bias and to exhibit a favourable safety profile, with fewer and milder side effects than BNT162b2.^{11,15}

The HIPRA-HH-4 trial conducted an in-depth, comprehensive immunogenicity evaluation and safety assessment of a PHH-1V booster in adults with chronic immunocompromising conditions who had completed a primary mRNA-based SARS-CoV-2 vaccination series. The selected study population included some of the most prevalent and vulnerable immunocompromising conditions for which boosters are recommended, given their varying degrees of humoral and cellular immune impairment. A thorough understanding of vaccine responsiveness and its durability over 12 months post-vaccination in these populations can guide optimal booster schedules and improve clinical outcomes in immunocompromised individuals, addressing limitations of previous studies.

Results

Participant disposition and baseline characteristics

A total of 240 participants were enrolled, of whom 238 received the study vaccine (intention-to-treat [ITT] and safety population [SP]) and were followed up on Days 14, 91, 182, and 365 post-vaccination, where applicable (**Figure 1a**). By the end-of-study visit, 225 participants (95%) had completed follow-up (**Figure 1b**). Ten participants were excluded from the immunogenicity analyses (5 had SARS-CoV-2 infection within 14 days post-vaccination, 4 did not meet the eligibility criteria, and 1 had an invalid baseline N serology result). The modified ITT (mITT) immunogenicity population comprised 228 participants: 59 (26%) people living with HIV (PWH), 35 (15%) kidney transplant (KTx) recipients, 55 (24%) participants on chronic haemodialysis/peritoneal dialysis (HD), 24 (11%) participants with primary antibody deficiency (PAD), and 55 (24%) participants with autoimmune disease (AID) receiving anti-CD20 therapy. A subset of 141 participants (~ 50% per group) was included in the T-cell immunogenicity population (IGP-T) analysis.

Baseline characteristics of the ITT population are shown in **Table 1**. The median age was 56 years (range 21–90). Age and sex assigned at birth varied by group: 59% of participants on HD were ≥ 65 years vs. 8% of PWH; 70% of participants with AID were female vs. 19% in the KTx group. The KTx and HD groups had the highest number of comorbidities associated with severe COVID-19, including hypertension, diabetes, chronic obstructive pulmonary disease, and cardiac disease. Overall, 53% of participants had confirmed prior SARS-CoV-2 infection. Participants in the PWH group had a median CD4⁺ T-cell count nadir of 62 (range 0–333) cells/mm³ and a median current CD4⁺ T-cell count of 240 (range 30–600) cells/mm³; all were on antiretroviral treatment (ART), with 9 (15%) participants having low-level viraemia (range 50–207 HIV-1 RNA copies/mL) at Day 0 and the remaining participants being virologically suppressed. Total CD3⁺ T-cell and CD19⁺ B-cell counts were largely preserved across groups, except in AID (median < 30 B cells mm³) due to anti-CD20 therapy.

Humoral response to the PHH-1V booster

For the primary immunogenicity endpoint, neutralising antibody titres against Omicron subvariants increased significantly by Day 14 in all groups except AID. **Table 2** and **Figure 2a** show the geometric mean titre (GMT) and geometric mean fold rise (GMFR) of neutralising antibodies determined by pseudovirus-based neutralisation assay (PBNA) against Omicron BA.1—or live-virus neutralisation assay (VNA) for the PWH group against Omicron BA.2—on Day 0 and Day 14. GMT increased from baseline to Day 14 in PWH (GMT 7,926 on Day 14; GMFR 4.49-fold), KTx (GMT 1,924; GMFR 3.18), HD (GMT 4,910; GMFR 7.77), and PAD

(GMT 527; GMFR 3.01) groups (all $P < 0.01$), whereas AID showed no significant increase (GMFR ~ 1.2 , $P > 0.05$).

We performed subgroup analyses to differentiate booster-induced responses in individuals with and without previous SARS-CoV-2 exposure. Participants in the PWH, KTx, and HD groups with prior infection had consistently higher neutralisation GMT values than those without prior infection at both Day 0 and Day 14. Nevertheless, significant titre increases were observed by Day 14 in both SARS-CoV-2 exposure subgroups (**Figure 2b**). Similar booster-induced increases were observed against other Omicron subvariants (BA.2 and BA.4/5), as well as against Beta (a vaccine-matched strain) and the ancestral WH1 D614G variant (**Supplementary Figure S1a-h**), indicating broadly cross-reactive humoral immunity induced by PHH-1V immunisation.

Anti-RBD binding IgG levels (determined by electrochemiluminescence assay) also increased significantly by Day 14 in all groups except AID (**Figure 2c-d**). A ≥ 2 -fold increase in anti-RBD binding antibody titres was observed in 93% (95% CI 84; 98) of PWH. Moreover, over 60% of individuals in the PWH and HD groups had a ≥ 4 -fold increase in anti-RBD binding GMT, compared with less than 35% in the KTx, PAD, and AID groups (**Table 2**). Binding antibody levels on Day 14 strongly correlated with neutralising antibody titres across all groups, as measured by VNA or PBNA ($\rho > 0.80$; $P < 0.0001$ for all groups) (**Figure 2e**).

Next, we evaluated the cross-reactivity potential of PHH-1V-induced antibodies against other human coronaviruses. A significant increase in IgG against SARS-CoV was observed between Day 0 and Day 14, with adjusted differences (95% CI) of 0.37 (0.30; 0.43) in PWH, 0.23 (0.11; 0.35) in KTx, 0.31 (0.23; 0.40) in HD, and 0.14 (0.06; 0.23) in PAD ($P < 0.01$ for all groups except AID) (**Figure 2f**). In contrast, PHH-1V booster vaccination did not increase cross-reactivity to any of the other human coronaviruses tested (HCoV-OC43, MERS-CoV, HCoV-HKU1, HCoV-229E, or HCoV-NL63, data not shown). IgG avidity indices increased from Day 0 to Day 14 (**Figure 2g**) and remained high through later follow-up visits, in line with parallel antibody affinity maturation.

Finally, IgG subclass profiles in response to booster vaccination were analysed. Following the PHH-1V booster, participants in the PWH, KTx, and HD groups exhibited a broad increase in IgG1, IgG2, IgG3, and IgG4 responses. In contrast, individuals with PAD showed a more restricted humoral response, characterised predominantly by significant increases in IgG1 and IgG3, with negligible induction of IgG2 and IgG4 isotypes. Participants with AID receiving anti-CD20 therapies showed only a slight increase in anti-RBD Omicron BA.1 IgG1 levels after PHH-1V booster immunisation (**Figure 2h**).

For longitudinal analyses beyond Day 14, data were censored at the latest available time point prior to either confirmed new SARS-CoV-2 infection, administration of a new booster vaccination, passive monoclonal antibody infusion, or replacement IgG therapy—the latter applicable to participants with PAD only. By Day 91, the neutralising antibody GMTs had declined from the Day 14 peak in all cohorts but remained significantly higher than baseline in PWH, KTx, and HD (**Figure 3a**). At Day 182, antibody levels waned further compared to Day 91, yet participants in the PWH and HD groups maintained significantly higher neutralising titres compared to baseline. By Day 365, none of the groups had significantly elevated neutralising titres compared to baseline. Participants with AID showed persistently low titres, with no significant change from baseline at any time point. A comparable trend in durability was observed for anti-RBD IgG binding: the PWH, KTx, and HD cohorts exhibited significantly elevated antibody levels until Day 182 relative to baseline, but these levels also reverted to pre-vaccination values by Day 365 (**Figure 3b**). Of note, antibody responses in participants with previous SARS-CoV-2 exposure declined more slowly: for example, in PWH with prior infection, the neutralising GMT at Day 365 was 2,878 (vs. 2,430 in those without prior infection). Despite quantitative waning of binding antibody titres, especially IgG1 (**Supplementary Fig. S2**), antibody avidity remained high at 1 year, suggesting maintenance of antibody quality (**Figure 3c**). In summary, the PHH-1V booster was effective in eliciting a significantly increased humoral immunity in most immunocompromised individuals, with durability at 12 months varying by condition (greatest in PWH and lowest in B-cell-depleted AID).

T-cell responses to the PHH-1V booster

RBD-specific T-cell responses were evaluated in 141 participants (IGP-T) by IFN- γ ELISpot and intracellular cytokine staining (ICS). At baseline, most participants exhibited detectable levels of RBD-specific IFN- γ^+ T cells against Omicron BA.1, regardless of their underlying condition. The adjusted treatment mean (95% CI) of RBD-specific T-cell frequencies at baseline was 77 (41; 126) IFN- γ^+ spot-forming cells (SFC)/ 10^6 peripheral blood mononuclear cells (PBMC) in PWH, 73 (34; 123) in KTx, 133 (57; 188) in HD, 162 (78; 277) in PAD, and 207 (140; 287) in AID. On Day 14, all groups showed numerically higher T-cell responses, with PWH and HD participants reaching significantly higher levels of RBD-specific IFN- γ^+ T cells against Omicron BA.1 RBD peptides ($P = 0.0283$ and $P = 0.0122$, respectively, **Figure 4a**). When stratified by previous SARS-CoV-2 exposure, as in the antibody analyses, RBD-specific T-cells were consistently higher in individuals with prior infection in all evaluable stratified groups (**Figure 4b**). Notably, participants with PAD and AID, despite weak antibody responses, showed preserved or even

higher frequencies of RBD-specific T cells before and after vaccination than other groups, and this was consistent across all tested variants (**Supplementary Figure S3a-d**).

We assessed T-cell response durability by ELISpot at later visits, with data censored at the latest available time point before confirmed new SARS-CoV-2 infection or administration of a subsequent booster vaccination. By Day 91, IFN- γ ELISpot responses showed a contraction from their Day 14 peak in all groups (**Figure 4c**).

Next, to assess the relationship between humoral and cellular immunity, we performed correlation analyses of these immune parameters in each immunocompromising condition. In the PWH group, we found a positive correlation between neutralising antibody levels and T-cell-mediated responses at Day 14 ($\rho = 0.50$; $P = 0.0063$) (**Figure 4d**). We observed no evident correlation between antigen-specific antibody levels measured in plasma and T-cell frequencies in PBMC in any of the other study groups. To further characterise the vaccine-induced T-cell responses, ICS was performed for IFN- γ and IL-2, along with staining for CD4 and CD8 T-cell lineage markers. These analyses indicated that the increases in IFN- γ^+ RBD-specific T cells were mainly driven by increases in antigen-specific CD4 $^+$ T cells (**Figure 5a-b**), while no significant changes were observed in the frequency of CD4 $^+$ T cells producing IL-2 or co-expressing IFN- γ and IL-2 across the different groups (**Figure 5c-f**).

Finally, immunogenicity outcomes were stratified by sex assigned at birth and by participants' age at study entry (< 65 or ≥ 65 years). At Day 14 post-booster vaccination, female participants exhibited significantly lower binding antibody levels than males ($P = 0.0250$, **Supplementary Figure S4a**); however, this difference was largely influenced by the overrepresentation of women (70%) in the AID cohort, which showed minimal to absent humoral response to vaccination. The observed differences were no longer significant after excluding participants with AID ($P > 0.05$ for all, data not shown). Age did not significantly affect neutralising or binding antibody titres post-vaccination. Nonetheless, individuals aged ≥ 65 years displayed significantly reduced frequencies of RBD-specific IFN- γ^+ T cells at Day 14 ($P = 0.0164$, **Supplementary Figure S4b**), and this difference remained significant even when participants on HD ≥ 65 years ($n = 35$, 59%) were excluded ($P = 0.0232$, data not shown). Pre-vaccination analyses revealed that total baseline B-cell counts were associated with higher post-vaccination neutralising and binding antibody titres, although the correlations were relatively weak ($r = 0.20$ and $r = 0.35$, $P < 0.01$ for both). In contrast, total T-cell counts were not associated with the magnitude of RBD-specific IFN- γ^+ T cell responses ($r = -0.07$, $P = 0.4049$, **Supplementary Figure S4c**). In PWH, neither humoral nor cellular responses showed significant associations with nadir CD4 $^+$ T-cell levels or absolute CD4 $^+$ T-cell counts at the time of vaccination. However, both post-vaccination neutralising antibody titres and frequencies of RBD-specific IFN- γ^+ T cells positively correlated with higher baseline

CD4:CD8 ratios ($r = 0.29$ and $r = 0.39$, respectively, $P < 0.05$ for both, **Supplementary Figure S5a-c**). In the AID cohort, antibody titres on Day 14 did not correlate with the time elapsed since the last rituximab administration to vaccination, underscoring the prolonged impact of B-cell-depleting therapies on vaccine-induced humoral immune responses (**Supplementary Figure S5d**).

Safety and tolerability

From Day 0 to Day 7, a total of 242 solicited local adverse events (AEs) were reported by 130 participants (55%), and 255 solicited systemic AEs were reported by 86 participants (36%), all of which were considered related to PHH-1V. Pain at the injection site was the most common local AE, while fatigue was the most frequently reported systemic AE across all groups (**Figure 6**). All local reactions were grade 1–2, except for erythema/redness, which was reported as grade 3 in 4 participants (1.68%). Grade 3 systemic events were reported only for fatigue (9 events in 2 [0.84%] participants), joint pain (1 event in 1 [0.42%] participant), myalgia (5 events in 2 [0.84%] participants), and malaise (3 events in 2 [0.84%] participants). Within the first 28 days after PHH-1V booster vaccination, a total of 580 treatment-emergent adverse events (TEAEs) were documented in 165 participants (69%), mostly of mild to moderate intensity (**Supplementary Table S1**). Three serious TEAEs were reported in 3 participants (1.26%), all unsolicited, and none was considered related to PHH-1V administration. These included a grade 3 limb cellulitis (KTx), a grade 3 pneumonia (HD), and a grade 4 acute myocardial infarction (PAD), all requiring hospitalisation.

Notably, participants from the AID and PAD groups reported more solicited local and systemic AEs, with higher intensity, whereas the HD group had the lowest incidence of total solicited AEs (43%) (**Figure 6**). After PHH-1V booster vaccination, no significant changes were observed in haematology or biochemistry laboratory results.

PHH-1V was safe through the 12-month follow-up across all study groups. Overall, 82 serious adverse events (SAEs) were reported in 23.95% of participants and 122 medically attended AEs (MAAEs) in 31.51% of participants (**Supplementary Table S2**). No SAEs or medically attended adverse events (MAAEs) were assessed as related to the study vaccine. Six participants from the HD group and one participant from the KTx group died during the study due to underlying disease or unrelated causes, rather than TEAEs. Causes of death included cardiogenic shock, progressive organ failure, exacerbated chronic obstructive pulmonary disease, sepsis, and renal transplantation with multiple complications; none was considered related to PHH-1V or COVID-19.

SARS-CoV-2 infections

Breakthrough SARS-CoV-2 infections ≥ 14 days after PHH-1V administration throughout the study period were documented in 61 participants—56 with non-severe infections—which mostly coincided with the 2022 local epidemic waves (**Figure 7a, b**). During the study, any SARS-CoV-2 infection requiring ≥ 24 hours of hospitalisation was classified as a severe COVID-19 episode, regardless of oxygen requirements. Overall, 5 (2.10%) participants had severe COVID-19 infections and were hospitalised (1 in KTx, 3 in HD, and 1 in AID). All 5 required non-invasive ventilation, and 1 KTx recipient required intensive care unit (ICU) admission, but all recovered before the end of the study. There were no deaths associated with COVID-19 (**Supplementary Table S3**).

Discussion

Data on booster vaccine responsiveness, durability, and safety in immunocompromised individuals remain limited. To address these gaps, HIPRA-HH-4 evaluated heterologous PHH-1V booster vaccination over 365 days in 240 adults across five clinically distinct immunocompromised cohorts. These longitudinal data provide deep insight into vaccine-induced immune responses, their durability, and safety, helping to inform optimal booster schedules and prevention strategies for vulnerable groups.

We report 365-day immunogenicity and safety results from heterologous PHH-1V booster vaccination in adults with diverse immunosuppressive conditions who had previously completed an mRNA SARS-CoV-2 primary vaccination course. PHH-1V is the first generation of an adjuvanted, protein-based recombinant heterodimer vaccine made from the RBD of the Alpha and Beta SARS-CoV-2 variants. A total of 240 participants from five clinically distinct cohorts with impaired immune function were enrolled in the HIPRA-HH-4 study, aiming to reflect the demographic and clinical diversity observed in immunocompromised populations.¹⁶ Overall, PHH-1V booster vaccination demonstrated a favourable safety profile and elicited humoral and cellular immune responses in most of the immunocompromised groups tested.

The PHH-1V booster demonstrated significant increases in anti-RBD binding antibody titres and neutralising activity from Day 0 to Day 14 against the SARS-CoV-2 Omicron variants BA.1, BA.2, and BA.4/5, which were the dominant subvariants circulating at the start of the trial in May 2022 (<https://gisaid.org/hcov19-variants/>) after replacing the Delta lineage. This response was consistent across study groups, except in participants with a severely impaired B-cell compartment, namely those with AID on chronic anti-CD20 therapy. This aligns with data from studies using rituximab and other anti-CD20 treatments, which show prolonged B-cell depletion and marked effects on vaccine-induced antibody production. Among participants without prior SARS-CoV-2 infection in the PWH, KTx, and HD groups, neutralising antibody GMTs at Day 14 were close to those observed in healthy adults in the HIPRA-HH-2 booster vaccination trial.¹¹ Although the development of vaccines to protect against newly emerging variants is needed, booster doses based on preceding variants can also enhance cross-reactive immunity, as seen with PHH-1V-induced cross-reactive responses against several Omicron subvariants.¹¹ We found a strong correlation between anti-RBD binding and neutralising antibody titres across groups against all variants tested in our study on Day 14. However, while total anti-RBD binding antibodies remain valuable for tracking humoral response dynamics, particularly in immunocompromised or other high-risk groups, total binding antibody assays should be interpreted with caution when guiding vaccination decisions, since the correlation between total

binding antibody titres and protection against Omicron and its subvariants has become far less predictable for newer immune escape mutations.¹⁷

Detailed analyses demonstrated cross-reactivity of the humoral response between SARS-CoV and SARS-CoV-2, reflecting the greater homology between the two viruses in the RBD region, but not with other human coronaviruses.¹⁸ Across all groups except AID, antibody avidity increased significantly following PHH-1V booster vaccination and was maintained over time, indicating ongoing humoral maturation. In the PAD group, the persistent rise in antibody titres and avidity during the study likely reflects the effects of chronic external IgG administration. This may not only provide individual immune protection in this group despite lower vaccine immunogenicity, but also reflect antibody maturation from continuous exposure to SARS-CoV-2 infections at the population level—*i.e.*, among plasma donors.

At Day 14, participants in the PWH, KTx, and HD groups exhibited a broad induction of IgG1, IgG2, IgG3, and IgG4 subclasses, supporting a favourable humoral response that may reflect preserved class-switching capacity and/or recall of RBD-specific memory B cells. Conversely, participants with PAD showed more restricted subclass breadth, with a predominance of IgG1 and IgG3 and minimal boosting of IgG2 and IgG4 responses, which could reflect known maturation defects in B-cell differentiation in PAD.¹⁸ Notably, participants with AID receiving anti-CD20 therapy exhibited modest increases in IgG1 only, suggesting a limited capacity for subclass diversification under conditions of B-cell depletion. These findings emphasise that the qualitative profile of antibody responses—beyond total IgG titres—is heavily shaped by the nature of immunosuppressive treatment or underlying disease, with important implications for booster vaccination, susceptibility to infections, and vaccine strategies targeting pathogens beyond SARS-CoV-2.

Despite some cross-variant immunity from existing first-generation vaccines, both the World Health Organisation (WHO) and the European Medicines Agency (EMA) emphasise the need for ongoing updates to COVID-19 vaccines as new variants emerge. While most guidelines prioritise immunocompromised individuals for booster doses, it is less clear whether the recommended interval should be 6 or 12 months after their last dose. In practice, this often translates into a single booster dose per year, coinciding with the flu vaccination campaign, to maintain higher protection during periods of anticipated high transmission. However, SARS-CoV-2 continues to circulate year-round at non-negligible levels and has not yet reached a seasonal pattern. In addition, we show that neutralising antibody responses declined substantially after their initial peak at Day 14 across most study groups, with strong detectable neutralisation not persisting beyond 6 months in most participants. These findings indicate that more frequent booster vaccinations, ideally 6-monthly, may help these individuals maintain optimal protection.

Beyond antibodies, immune protection is multifaceted and involves memory B and T cells, among other factors. RBD-specific T cells were detected in most participants at study entry, reflecting immunological memory established during primary vaccination and/or past SARS-CoV-2 infection. RBD-specific T cells significantly increased on Day 14 after PHH-1V in PWH and participants on HD, then contracted. RBD-specific T cells were predominantly driven by IFN- γ -producing CD4⁺ T cells, with minimal involvement of CD8⁺ T cells, at least in the peripheral blood. These Th1-polarised responses are consistent with previous data on PHH-1V in non-immunocompromised individuals,¹¹ and show a remarkable ability of PHH-1V to elicit these responses in the PWH and HD groups.

Overall, post-vaccination humoral responses appeared largely unaffected by factors such as age or sex, suggesting that underlying immunocompromising conditions and/or treatments play a more critical role in antibody vaccine responsiveness within these populations. However, the frequencies of post-vaccination T-cell responses were significantly lower among participants aged 65 years and older, which aligns with the well-documented relationship between age-associated immune senescence, overall reduced vaccine responsiveness, and COVID-19 severity.¹⁹

The different immunological outcomes of PHH-1V in our study reflect the specific underlying immunocompromising conditions in each group. Despite low nadir CD4 and suboptimal immune recovery under antiretroviral treatment, PWH showed a unique coordination between humoral and cellular response to PHH-1V booster vaccination²⁰. Notably, post-vaccination levels of both humoral and cellular responses correlated more strongly with the baseline CD4:CD8 ratio than with absolute CD4⁺ T cell counts. This supports a growing body of literature highlighting the relevance of the CD4:CD8 ratio²¹ as a more accurate biomarker of T-cell functional status. These findings also suggest that immunisation should not be delayed solely based on CD4⁺ counts and that vaccines should be administered as soon as PWH achieve HIV suppression, underscoring the importance of early and effective antiretroviral therapy initiation.²⁰

The KTx recipients exhibited robust humoral but limited T-cell responses, likely attributable to chronic anti-rejection regimens, including corticosteroids, calcineurin inhibitors (tacrolimus and ciclosporin) and mycophenolate mofetil.²² While the lack of increases in T-cell responses might result from the compartmentalisation of T cells, located mainly in secondary lymphoid tissues, these findings are consistent with poorer clinical outcomes and delayed viral clearance observed in these individuals.²³ These findings also underscore the need for tailored vaccination strategies in transplant recipients, potentially including additional booster doses, as well as the development of adjunctive immunotherapies and antivirals to enhance viral clearance and ultimately reduce the risk of severe disease in this population.

Participants in the HD group showed neutralising antibody responses after PHH-1V that were comparable to those of the healthy adult population¹¹, despite advanced age and a high comorbidity burden. Booster doses in patients on HD have been shown to sustain humoral responses and enhance T-cell activity. Given the frequent visits to healthcare centres and associated risks in this population, continued prioritisation for booster vaccination is warranted.^{24,25} Moreover, haemodialysis-related oxidative stress and chronic inflammation may impair immune responses, underscoring the importance of booster-induced immunity.^{26,27}

Participants in the PAD group displayed more variable immune responses, likely reflecting the wide spectrum of antibody production defects among these populations.^{28–31} Of note, although vaccine-induced humoral responses were modest, receiving immunoglobulin replacement therapy may provide passive protection against SARS-CoV-2 acquisition in this group. However, efficacy likely varies with donor exposure to earlier SARS-CoV-2 variants and antibody levels.^{32–34} Although total vaccine-specific T-cell responses were not significantly increased after vaccination in participants with PAD—probably due to limited sample size—frequencies of RBD-specific IFN- γ ⁺ T-cells were among the highest in the study, suggesting a compensatory mechanism for impaired humoral immunity. As in the PAD cohort, participants with AID treated with anti-CD20 therapies showed no antibody induction, but T-cell responses were preserved before and after booster vaccination. This supports the idea that vaccines capable of eliciting or boosting cellular immunity, even in the presence of limited humoral responses, may confer added benefits in PAD and AID, thereby enhancing protection against severe COVID-19, as recently described for vaccines against Orthoflavivirus,³⁵ and highlights an adaptive shift towards T-cell-mediated protection.^{40,37}

The time since last rituximab administration did not correlate with post-vaccination antibody titres, confirming the durability of B-cell depletion. In contrast to PAD, individuals with AID do not regularly receive immunoglobulin replacement therapy as part of their treatment. Therefore, tailored pre-exposure prophylaxis using monoclonal antibodies may help bridge protection gaps in this group.

PHH-1V booster was confirmed to have a favourable safety profile across all groups of immunocompromising conditions. Importantly, PHH-1V safety was also confirmed during long-term follow-up through 12 months post-vaccination, with no related MAAEs or SAEs reported beyond Day 14 among the 225 participants who completed 1 year of follow-up (95% retention). Interestingly, increased reactogenicity was observed in both the PAD and AID groups, reflected by a higher frequency and intensity of AEs compared with other cohorts, albeit still lower than levels typically reported following mRNA-based vaccination.³⁸ Notably, individuals in the PAD and AID groups were those with more limited or absent induction of vaccine-induced antibodies,

challenging the commonly assumed association between vaccine reactogenicity and immunogenicity for B-cell responses; as in these cases, reactogenicity may reflect innate immune mechanisms or delayed type IV hypersensitivity reactions mediated by CD4⁺ T cells.³⁹

Our study reported 61 cases of SARS-CoV-2 breakthrough infections, most of which were mild-to-moderate COVID-19. This number coincided with the reported immune-escaped Omicron subvariant waves circulating during the 2022–2023, which transitioned from Omicron B.1.1.529 and BA to Omicron XBB subvariants (<https://gisaid.org/hcov19-variants/>). Interestingly, ART-suppressed PWH, even with low CD4 T-cell counts, had the lowest incidence and the least severe cases among all cohorts of immunosuppressed individuals in the HIPRA-HH-4 trial. While the AID, PAD, and HD groups had a higher incidence of SARS-CoV-2 infections, the only 5 cases of severe COVID-19 requiring hospitalisation and/or ventilation were concentrated within the HD and KTx groups, reflecting older participants, with higher comorbidities and more T-cell immunosuppression, as well as the need for better antiviral treatments.

To our knowledge, HIPRA-HH-4 is among the few international COVID-19 booster vaccine interventional studies to include such broad clinical diversity of immunocompromising conditions, together with a highly comprehensive immunological characterisation of humoral and cellular responses. Nevertheless, the study has some limitations. The small sample sizes in some subgroups limited the power of statistical analyses, such as those assessing age- or sex-specific effects. Other high-risk groups, such as haematopoietic stem cell transplant recipients, patients with active cancer on chemotherapy, or individuals with Long COVID, were not evaluated here. In addition, the study's focus on immune responses to Omicron subvariants circulating during the 2022–2023 period—when the trial was conducted—may limit generalisability to newer, emerging variants. However, the observed ranges of cross-reactivity across immunogenicity studies support the broader applicability of the more contemporaneous adapted recombinant protein vaccine versions.

In conclusion, this study provides evidence that the PHH-1V recombinant protein-based vaccination is safe, well-tolerated, and immunogenic in individuals with several chronic immunocompromising conditions who were previously vaccinated with mRNA SARS-CoV-2 vaccines, supporting its use as a heterologous booster vaccine in these especially vulnerable populations. It effectively enhanced humoral immune responses across most groups except in individuals with AID receiving chronic B-cell depletion therapies, with increases or preservation of T-cell immunity seen even in the absence of antibody responses. The HIPRA-HH-4 study, extending to 12 months post-booster vaccination, provides relevant information on the durability of humoral and cellular responses, and on the incidence and severity of SARS-CoV-2 breakthrough infections across different groups with immunocompromising conditions. These

461 findings help guide tailored prevention strategies, such as the potential benefit of 6-monthly
462 booster vaccinations in the HD and KTx groups and support the development of new monoclonal
463 antibodies for passive pre-exposure prophylaxis for individuals such as those with AID receiving
464 chronic B-cell depletion treatments. Moreover, these findings reinforce the need to include
465 immunocompromised individuals in vaccine trials to tailor immune protection strategies for more
466 vulnerable populations.

Methods

Study design and participants

HIPRA-HH-4 (NCT05303402) was a Phase IIb/III, open-label, single-arm, multicentre trial designed to evaluate the immunogenicity, safety, and tolerability of a booster dose of PHH-1V, enrolling 240 individuals with pre-existing immunocompromising conditions at three sites in Spain and three in Turkey. First Participant First Visit (FPFV) and Last Participant Last Visit (LPLV) were conducted on 12th May 2022 and 9th January 2024, respectively.

Eligible participants were adults (≥ 18 years) who had completed primary immunisation with COVID-19 vaccines and had one of the following conditions: a) HIV infection with CD4⁺ T-cell counts < 400 cells/mm³ within 6 months before inclusion (PWH); b) > 1 year post-kidney transplant and currently receiving ≥ 3 immunosuppressive drugs including tacrolimus/cyclosporine, glucocorticoids, and mycophenolate or everolimus/sirolimus (KTx); c) end-stage kidney disease on haemodialysis or peritoneal dialysis for ≥ 6 months (HD); d) any primary antibody deficiency and on chronic IgG replacement therapy for ≥ 6 months (PAD); e) chronic autoimmune diseases, including multiple sclerosis (MS), receiving any anti-CD20 therapy (*e.g.*, rituximab/ocrelizumab) within 6 months and > 14 days before inclusion (AID). Complete primary vaccination was defined according to the country-specific vaccination schedules. In Spain, participants were required to have received either three doses of an mRNA-based SARS-CoV-2 vaccine (BNT162b2 [Comirnaty[®]; Pfizer-BioNTech] and/or mRNA-1273 [Spikevax[®]; Moderna]) or two doses plus documented history of SARS-CoV-2 infection, with the last vaccine dose and/or infection occurring ≥ 91 days before the baseline visit. For participants included in Turkey, participants were required to have completed a primary vaccination schedule with BNT162b2 and/or CoronaVac (CoronaVac[®]; Sinovac), with or without additional booster doses of the same vaccines, and to have received the last vaccine dose ≥ 91 days before baseline. Participants with prior SARS-CoV-2 infection were eligible provided that the infection, confirmed by rapid antigen test (RAT), polymerase chain reaction (PCR), and/or nucleocapsid (N)-specific serology, had occurred ≥ 91 days before baseline. Those requiring hospitalisation for prior SARS-CoV-2 infection had to have been discharged > 30 days before inclusion and have no evidence of post-acute sequelae of SARS-CoV-2 infection (PASC/Long COVID). Full eligibility criteria and study populations are provided in the **Supplementary Material**. Additional study design information is available in the Study Protocol (**Supplementary Data 1**) and in the Statistical Analysis plan (**Supplementary Data 2**). After trial initiation, eligibility criteria for KTx and AID participants were clarified and broadened. The primary immunogenicity endpoint was restricted to Omicron BA.1—with Omicron BA.2 assessed by VNA in the PWH group—while other variants were moved to secondary endpoints following regulatory feedback; the

planned Day 14 interim analysis was replaced by Day 91; and N-protein serology was added to identify intercurrent SARS-CoV-2 infections. Turkish vaccination eligibility was broadened to reflect widespread booster uptake, and the Day 365 visit was subsequently removed for Turkish participants because of very low recruitment (**Supplementary Data 1**).

The trial was approved by the Ethical Review Committee of Hospital Clínic in Barcelona (Spain) and by the Spanish and Turkish regulatory authorities and was conducted in accordance with the ethical principles of the Declaration of Helsinki and the ICH Harmonised Tripartite Guideline E6(R2): Good Clinical Practice (GCP). The biological biosafety committee of the Research Institute Germans Trias i Pujol (Spain) approved SARS-CoV-2 *in vitro* experiments at the BSL3 laboratory (CSB-20-015-M7). All participants provided written informed consent before enrolment. A Community Advisory Panel reviewed study materials, provided input on participant outreach strategies, and performed external oversight throughout the entire trial development. Materials developed under the RBDCOV project are available at <https://www.eatg.org/rbdcov-community-engagement-toolbox/>

Procedures

On Day 0 (baseline), all participants received a single 40 µg dose of PHH-1V via intramuscular injection into the non-dominant upper arm, followed by a 15–30-minute observation period based on allergy history. The PHH-1V vaccine was adjuvanted with an oil-in-water emulsion based on squalene (SQBA), developed and manufactured by HIPRA Group, and it was provided in ready-to-use multidose vials containing 10 doses of 0.5 mL (40 µg of recombinant protein [selvacovatein, active substance]) and stored at 2–8 °C. Follow-up on-site visits for immunogenicity and safety evaluations were conducted on Days 14, 91, and 182 for all participants, with an additional Day 365 visit for participants in Spain only. A subset of participants consented to undergo larger-volume blood sampling for T-cell immune analyses at the same visits (**Fig. 1b**).

Study variables and outcomes

Humoral and cellular responses

HIPRA-HH-4 primary endpoints were neutralising antibody levels against Omicron BA.1—or against Omicron BA.2 for the PWH group (**Supplementary Methods**)—expressed as GMT before and 14 days after PHH-1V vaccination, and the geometric mean fold rise (GMFR) from Day 0 to Day 14. Secondary endpoints included neutralisation against Omicron BA.1 and other Omicron subvariants (BA.2 and BA.4/5), and against Beta and WH1 D614G variants from Day 0 to Day 365. Neutralising antibody titres were measured by PBNA or VNA (PWH). Total

binding antibodies against the spike RBD were quantified by electrochemiluminescence immunoassay (ECLIA, Elecsys® Anti-SARS-CoV-2 S, Roche Diagnostics, Switzerland). Further details are provided in the **Supplementary Methods**.

IgG subclass levels (IgG1, IgG2, IgG3, and IgG4) to SARS-CoV-2 RBD Omicron BA.1 and total IgG cross-reactivity with the spike glycoproteins of related coronaviruses (SARS-CoV, hCoV-OC43, MERS-CoV, hCoV-HKU1, hCoV-229E, and hCoV-NL63) were determined as exploratory endpoints using a customised multiplex bead-based Luminex-like immunoassay, developed at IrsiCaixa. Beads coated with the corresponding RBD or spike variants were incubated with plasma samples collected on Day 0 and Day 14. IgG-bound antibodies were detected using a cocktail of biotinylated detection antibodies and a streptavidin-phycoerythrin conjugate. Mouse anti-human IgK, bovine serum albumin, and a pool of plasma samples collected before the COVID-19 pandemic served as positive and negative controls, respectively.

To determine the avidity of anti-RBD variant-specific IgG, one thousand beads of each antigen were incubated with diluted plasma samples and incubated at room temperature (RT) for one hour with shaking at 575 rpm. After washing, samples were incubated with either 2 M guanidine hydrochloride or PBS for 15 minutes at RT to allow antibody dissociation. Next, beads were washed and incubated with goat anti-IgG PE at 1/1000 (Jackson ImmunoResearch Laboratories, Inc., PA, USA) for one hour at RT with shaking. Finally, beads were washed and resuspended in 1x PBS. Samples were analysed using a Luminex100 reader (Luminex, DiaSorin Iberia S.A., Spain). The avidity index of each sample was calculated as the ratio between the mean signal obtained with and without guanidine treatment.

RBD-specific T-cell responses were measured in cryopreserved PBMCs using an IFN- γ -detecting enzyme-linked immunospot (IFN- γ ELISpot) assay at all time points, as well as ICS upon peptide pool stimulation as previously described¹¹ at baseline and at Day 14 post-vaccination. **Supplementary Figure S6** shows the gating strategy, and **Supplementary Table S4** shows the antibody panel.

Safety and tolerability

Participants recorded solicited local and systemic reactions using diary cards for 7 days following the PHH-1V booster vaccination. AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 5.0, and specified as related or unrelated to vaccination by the investigator. TEAEs were defined as events occurring from vaccine administration through Day 28, or events present at baseline that worsened or were deemed vaccine-related. SAEs and MAAEs were documented until study end. Grade 3 and 4 laboratory parameter changes, as well as the incidence and severity of SARS-CoV-2 infections

occurring ≥ 14 days post-vaccination, were recorded. Medical history and AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 26.1, and concomitant medications were coded using the WHODrug Global dictionary, version B3.

Confirmed cases of SARS-CoV-2 infection ≥ 14 days after PHH-1V administration were recorded if positive by RAT or PCR. We defined severe COVID-19 infections as confirmed SARS-CoV-2 infections requiring hospitalisation. We recorded the number and percentage of ICU admissions, non-invasive ventilation administration, and COVID-19-associated deaths through the end of the study.

Statistical analysis

No formal sample size calculation was performed for this exploratory phase IIb/III study. Among the 240 participants enrolled, there was a 91% probability of observing at least one AE and a 69% probability of observing at least two AEs, assuming a true event rate of 1%. An interim analysis was performed after Day 91, once humoral immunogenicity and safety data were available for all participants enrolled in Spain. Early study termination was permitted for unacceptable participant risk, suboptimal recruitment, protocol non-compliance, insufficient evaluable data, or regulatory request (**Supplementary Data 1**).

A mixed-effects model for repeated measures (MMRM) was used to assess immunogenicity endpoints. Fixed effects included age group (≥ 18 to < 65 years; ≥ 65 years), visit number, Prior-CoV-2 (yes/no) and Prior/No Prior-CoV-2 subgroup-by-visit interaction. The repeated-measures structure corresponded to the visits for each participant. Log₁₀ transformation was considered for humoral data and square root transformation, for cellular T-cell response data, with values back-transformed for reporting. Humoral outcomes from these fitted models were reported as GMT with 95% confidence intervals (CI), whereas cellular outcomes were reported as back-transformed adjusted means with 95% CI. GMFR was analysed using ANOVA on log-transformed data, obtained as the ratio of the Day 14/Day 0 titres. Prior/No prior SARS-CoV-2 infection subgroup analyses were performed except in the PAD group, where SARS-CoV-2 N-serology is unable to distinguish between antibodies originating from external IgG replacement therapy and those from previous infections. The percentage of participants with ≥ 2 -fold and ≥ 4 -fold change in total binding antibody titre from baseline to Day 14 and their exact CI were estimated by the Clopper-Pearson method.

Safety analyses were performed in the SP. Immunogenicity analyses were performed using data from participants in the ITT population who met all eligibility criteria, had no incident SARS-CoV-2 infection before Day 14, and had a valid N-serology at baseline (mITT). Cellular immunogenicity analyses were performed in a subset of mITT (*i.e.*, IGP-T) with available

PBMCs. Missing data were not imputed. Other statistical tests were also applied, including the Mann-Whitney test, proportions estimated using the Clopper-Pearson method, and Spearman's and Pearson's correlations. Statistical analyses and visualisations were performed using SAS Version 9.4, R Version 4.3, and GraphPad Prism 10.4.2 (or later). All performed analyses matched the pre-specified Statistical Analysis Plan (**Supplementary Data 2**).

Role of the funding source

The European Union's Horizon Europe programme (grant agreement No. 101046118) and the Centre for the Development of Industrial Technology (IDI-20230889), under the Spanish Ministry of Science and Innovation, had no role in the analyses or reporting of the results. HIPRA provided the investigational drug, was involved in the study design, oversaw all safety monitoring activities, data analysis, and interpretation, and reviewed the manuscript.

References

1. Barnes, E. *et al.* SARS-CoV-2-specific immune responses and clinical outcomes after COVID-19 vaccination in patients with immune-suppressive disease. *Nat. Med.* **29**, 1760–1774 (2023).
2. Leston, M. *et al.* Disparities in COVID-19 mortality amongst the immunosuppressed: A systematic review and meta-analysis for enhanced disease surveillance. *Journal of Infection* **88**, 106110 (2024).
3. Evans, R. A. *et al.* Impact of COVID-19 on immunocompromised populations during the Omicron era: insights from the observational population-based INFORM study. *The Lancet Regional Health - Europe* **35**, (2023).
4. Williamson, E. J. *et al.* Factors associated with COVID-19-related death using OpenSAFELY. *Nature* **584**, 430–436 (2020).
5. Raglow, Z. *et al.* SARS-CoV-2 shedding and evolution in patients who were immunocompromised during the omicron period: a multicentre, prospective analysis. *Lancet Microbe* **5**, e235–e246 (2024).
6. Corey, L. *et al.* SARS-CoV-2 Variants in Patients with Immunosuppression. *New England Journal of Medicine* **385**, 562–566 (2021).
7. Parker, E. P. K. *et al.* Response to additional COVID-19 vaccine doses in people who are immunocompromised: a rapid review. *Lancet Glob. Health* **10**, e326–e328 (2022).

- 633 8. World Health Organization (WHO). COVID-19 advice for the public: Getting vaccinated.
634 *Update 8th October 2024* [https://www.who.int/emergencies/diseases/novel-coronavirus-](https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines/advice)
635 [2019/covid-19-vaccines/advice](https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines/advice) (2024).
- 636 9. Shoham, S. *et al.* Vaccines and therapeutics for immunocompromised patients with
637 COVID-19. *EClinicalMedicine* **59**, (2023).
- 638 10. European Medicines Agency. *Bimervax: EPAR - Product Information*. (2023).
- 639 11. Corominas, J. *et al.* Safety and immunogenicity of the protein-based PHH-1V compared
640 to BNT162b2 as a heterologous SARS-CoV-2 booster vaccine in adults vaccinated against
641 COVID-19: a multicentre, randomised, double-blind, non-inferiority phase IIb trial. *The*
642 *Lancet Regional Health - Europe* **28**, 100613 (2023).
- 643 12. Corominas, J. *et al.* Humoral and cellular immune responses after 6 months of a
644 heterologous SARS-CoV-2 booster with the protein-based PHH-1V vaccine in a phase IIb
645 trial. *Vaccine* **47**, 126685 (2025).
- 646 13. Borralleras, C. *et al.* The PHH-1V HIPRA vaccine: a new tool in the vaccination strategy
647 against COVID-19. *Rev. Esp. Quimioter.* **36**, 507–515 (2023).
- 648 14. Blanco, J., Trinité, B. & Puig-Barberà, J. Rethinking Optimal Immunogens to Face SARS-
649 CoV-2 Evolution Through Vaccination. *Influenza Other Respir. Viruses* **19**, (2025).
- 650 15. Aleebrahim-Dehkordi, E. *et al.* T helper type (Th1/Th2) responses to SARS-CoV-2 and
651 influenza A (H1N1) virus: From cytokines produced to immune responses. *Transpl.*
652 *Immunol.* **70**, 101495 (2022).
- 653 16. Prasad, V. & Makary, M. A. An Evidence-Based Approach to Covid-19 Vaccination. *New*
654 *England Journal of Medicine* <https://doi.org/10.1056/NEJMs2506929> (2025)
655 [doi:10.1056/NEJMs2506929](https://doi.org/10.1056/NEJMs2506929).
- 656 17. Sánchez-Simarro, Á. *et al.* Estimating SARS-CoV-2 Omicron XBB.1.5 Spike-Directed
657 Functional Antibody Levels From an Anti-Receptor Binding Domain Wuhan-Hu-1-Based
658 Commercial Immunoassay Results. *J. Med. Virol.* **97**, (2025).
- 659 18. Shin, J. J. *et al.* Defining Clinical and Immunological Predictors of Poor Immune
660 Responses to COVID-19 mRNA Vaccines in Patients with Primary Antibody Deficiency.
661 *J. Clin. Immunol.* **42**, 1137–1150 (2022).

- 662 19. Jo, N. *et al.* Impaired CD4⁺ T cell response in older adults is associated with reduced
663 immunogenicity and reactogenicity of mRNA COVID-19 vaccination. *Nat. Aging* **3**, 82–
664 92 (2023).
- 665 20. Woldemeskel, B. A. *et al.* The BNT162b2 mRNA Vaccine Elicits Robust Humoral and
666 Cellular Immune Responses in People Living with Human Immunodeficiency Virus
667 (HIV). *Clinical Infectious Diseases* **74**, 1268–1270 (2022).
- 668 21. Ron, R. *et al.* CD4/CD8 Ratio During Human Immunodeficiency Virus Treatment: Time
669 for Routine Monitoring? *Clinical Infectious Diseases* **76**, 1688–1696 (2023).
- 670 22. Kolla, E. *et al.* COVID-19 Hospitalization in Solid Organ Transplant Recipients
671 on Immunosuppressive Therapy. *JAMA Netw. Open* **6**, e2342006 (2023).
- 672 23. Werbel, W. A. *et al.* Persistent SARS-CoV-2-specific immune defects in kidney
673 transplant recipients following third mRNA vaccine dose. *American Journal of*
674 *Transplantation* **23**, 744–758 (2023).
- 675 24. Davidovic, T. *et al.* Humoral and Cellular Immune Response After a 3-Dose Heterologous
676 SARS-CoV-2 Vaccination Using the mRNA-BNT162b2 and Viral Vector Ad26COVS1
677 Vaccine in Hemodialysis Patients. *Front. Immunol.* **13**, 907615 (2022).
- 678 25. Bathish, Y. *et al.* B and T cell responses to the 3rd and 4th dose of the BNT162b2 vaccine
679 in dialysis patients. *Hum. Vaccin. Immunother.* **20**, 2292376 (2024).
- 680 26. Donadei, C. *et al.* Impact of Single Hemodialysis Treatment on immune Cell
681 Subpopulations. *Journal of Clinical Medicine* vol. 12 Preprint at
682 <https://doi.org/10.3390/jcm12093107> (2023).
- 683 27. Campo, S., Lacquaniti, A., Trombetta, D., Smeriglio, A. & Monardo, P. Immune System
684 Dysfunction and Inflammation in Hemodialysis Patients: Two Sides of the Same Coin. *J.*
685 *Clin. Med.* **11**, (2022).
- 686 28. Gupta, S. *et al.* SARS-CoV-2-Specific and Functional Cytotoxic CD8 Cells in Primary
687 Antibody Deficiency: Natural Infection and Response to Vaccine. *J. Clin. Immunol.* **42**,
688 914–922 (2022).
- 689 29. Salinas, A. F. *et al.* SARS-CoV-2 Vaccine Induced Atypical Immune Responses in
690 Antibody Defects: Everybody Does their Best. *J. Clin. Immunol.* **41**, 1709–1722 (2021).

30. Ainsua-Enrich, E. *et al.* Kinetics of immune responses elicited after three mRNA COVID-19 vaccine doses in predominantly antibody-deficient individuals. *iScience* **25**, 105455 (2022).
31. Bracke, C. *et al.* Correlation between Clinical and Immunological Variables and Humoral Response to SARS-CoV-2 Vaccination in Adult Patients with Antibody Deficiency Disorders. *Pathogens* **11**, (2022).
32. Chang-Rabley, E., van Zelm, M. C., Ricotta, E. E. & Edwards, E. S. J. An Overview of the Strategies to Boost SARS-CoV-2-Specific Immunity in People with Inborn Errors of Immunity. *Vaccines (Basel)*. **12**, 675 (2024).
33. Cusa, G., Sardella, G., Garzi, G., Firinu, D. & Milito, C. SARS-CoV-2 vaccination in primary antibody deficiencies: an overview on efficacy, immunogenicity, durability of immune response and safety. *Curr. Opin. Allergy Clin. Immunol.* **24**, 37–43 (2024).
34. Mizera, D. *et al.* Cellular immune response to SARS-CoV-2 in patients with primary antibody deficiencies. *Front. Immunol.* **14**, 1275892 (2023).
35. Kalimuddin, S. *et al.* Vaccine-induced T cell responses control Orthoflavivirus challenge infection without neutralizing antibodies in humans. *Nat. Microbiol.* **10**, 374–387 (2025).
36. Zabalza, A. *et al.* Is humoral and cellular response to SARS-CoV-2 vaccine modified by DMT in patients with multiple sclerosis and other autoimmune diseases? *Mult. Scler.* **28**, 1138–1145 (2022).
37. Koehm, M. *et al.* Impact of different classes of immune-modulating treatments on B cell-related and T cell-related immune response before and after COVID-19 booster vaccination in patients with immune-mediated diseases and primary immunodeficiency: a cohort study. *RMD Open* **9**, (2023).
38. Yang, M. M. *et al.* Reactogenicity of the Messenger -RNA SARS-CoV-2 Vaccines Associated With Immunogenicity in Patients With Autoimmune and Inflammatory Disease. *Arthritis Care Res. (Hoboken)*. **74**, 1953–1960 (2022).
39. Lim, S. Y. *et al.* Correlation between Reactogenicity and Immunogenicity after the ChAdOx1 nCoV-19 and BNT162b2 mRNA Vaccination. *Immune Netw.* **21**, (2021).

Figure legends

Figure 1 | HIPRA-HH-4 trial design and participant disposition.

(a) Trial schedule, study visits (days), and assessments. Screening and Day 0 visits were allowed to be performed on the same day. At Day 0, all participants received a single dose of PHH-1V via intramuscular injection in the non-dominant upper arm, followed by an immediate observation period of 15 to 30 minutes, based on the participant's allergy history. Follow-up visits were performed on Day 14, Day 91, Day 182, and Day 365; the Day 365 visit was not included for individuals recruited at sites in Turkey. (b) CONSORT flow diagram for the trial. Figure created in BioRender. Mothe, B. (2026) <https://BioRender.com/eja2v1j>.

AEs, adverse events; AESIs, AEs of special interest; AID, participants with autoimmune disease receiving anti-CD20 therapy; CONSORT, Consolidated Standards of Reporting Trials; EOS, end of study; HD, participants on chronic haemodialysis/peritoneal dialysis; IC/EC, inclusion criteria/exclusion criteria; IGP-T, cellular immunogenicity population; ITT, intention-to-treat; KTx, kidney transplant recipients; LOFU, loss to follow-up; MAAEs, medically attended adverse events; mITT, modified ITT; PAD, participants with primary antibody deficiency; PWH, people with HIV; SAEs, serious adverse events; SP, safety population, TEAEs, treatment-emergent adverse events (i.e., AEs occurring within 28 days post-vaccination).

Figure 2 | Humoral response against Omicron BA.1/BA.2.

(a) Neutralising antibodies against Omicron BA.1 by PBNA, or against Omicron BA.2 by VNA for the PWH group, at Day 0 and Day 14 for each immunocompromising condition; and (b) stratified by previous SARS-CoV-2 exposure (“Prior” vs “No Prior”). (c) Anti-RBD binding antibody levels by immunocompromising condition group; and (d) stratified by previous infection. For the PAD group, stratification by previous SARS-CoV-2 exposure based on N-serology was limited by IgG replacement therapy and is therefore not shown. Individual determinations are shown as grey dots, and the bars represent the GMT, with its 95% CI indicated by horizontal black lines. GMT and 95% CI values were obtained by fitting an MMRM to log-transformed data. The shaded grey area depicts the limit of detection for each assay; *i.e.*, ID₅₀ = 20 reciprocal serum dilution for neutralisation and 0.40 U/mL for binding assays. The right-hand box in each panel shows the GMFR (95% CI) from Day 0 to 14, calculated by ANOVA using log-transformed data (solid lines, prior SARS-CoV-2 exposure; dashed lines, no prior SARS-CoV-2 exposure). (e) Spearman’s correlation (ρ) between binding antibody and neutralising antibody levels at Day 14 for each group. (f) Cross-reactivity with SARS-CoV; (g) avidity index; and (h) IgG subclass determinations against Omicron BA.1. All analyses were performed in the mITT population (N = 228) with available participant data and including only subgroups of > 10 participants. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

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; ID₅₀, 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 deficiency; PBNA, pseudovirus-based neutralisation assay; Prior, participants with prior exposure to SARS-CoV-2; PWH, people with HIV; RBD, receptor-binding domain; VNA, virus neutralisation assay.

Figure 3 | Durability of the humoral response against Omicron BA.1/BA.2.

(a) Neutralising antibodies against Omicron BA.1 by PBNA (or against Omicron BA.2 by VNA for PWH), (b) anti-RBD binding antibodies, and (c) the avidity index are shown over time for each immunocompromising condition. Beyond Day 14, data were censored at the latest available time point prior to 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, data beyond Day 14 are shown as dashed lines, as participants received chronic replacement IgG therapy every 3 weeks. For (a) and (b), the shaded grey area depicts the limit of detection for each assay. Each graph shows the number of participants with available data. * $P < 0.05$ for the last time point in which significant differences were detected.

AID, participants with autoimmune disease receiving anti-CD20 therapy; CI, confidence interval; GMT, geometric mean titre; HD, participants on chronic haemodialysis/peritoneal dialysis; KTx, kidney transplant recipients; PAD, participants with primary antibody deficiency; PBNA, pseudovirus-based neutralisation assay; PWH, people with HIV; RBD, receptor-binding domain; VNA, virus neutralisation assay.

Figure 4 | Cellular response against Omicron BA.1 by IFN- γ ELISpot assay.

(a) Magnitude of RBD-specific T-cell responses measured by IFN- γ ELISpot assay at Day 0 and Day 14, (b) stratified by previous SARS-CoV-2 exposure, and (c) shown over time for each immunocompromising condition. Beyond Day 14, data were censored at the latest available time point prior to either confirmed new SARS-CoV-2 infection and/or administration of a new booster vaccination. The number of participants with available data at each time point is shown. For PAD, stratification by previous SARS-CoV-2 exposure by N-serology results was limited by IgG replacement therapy and is therefore not shown. Individual determinations are shown as grey dots, and the bars represent the back-transformed adjusted mean and 95% CI values calculated by an MMRM using square-root-transformed data. (d) Spearman's correlation (ρ) between neutralising antibody levels and frequencies of IFN- γ^+ T-cell responses at Day 14 for each group. All analyses were performed with available data from participants in the IGP-T (N = 141) and including only subgroups of > 10 participants. * $P < 0.05$; ** $P < 0.01$.

AID, participants with autoimmune disease receiving anti-CD20 therapy; CI, confidence interval; ELISpot, enzyme-linked immunosorbent spot; GMT, geometric mean titre; HD, participants on chronic haemodialysis/peritoneal dialysis; 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; Prior, prior exposure to SARS-CoV-2; PWH, people with HIV; RBD, receptor-binding domain; SFC, spot-forming cells.

Figure 5 | Cellular response against Omicron BA.1 by ICS.

Frequencies of RBD-specific (a) IFN- γ^+ CD4 $^+$, (b) IFN- γ^+ CD8 $^+$, (c) IL-2 $^+$ CD4 $^+$, (d) IL-2 $^+$ CD8 $^+$, (e) IFN- γ^+ /IL2 $^+$ CD4 $^+$, and (f) IFN- γ^+ /IL2 $^+$ CD8 $^+$ T cells were measured by ICS at Day 0 and Day 14 for each immunocompromised condition. Individual determinations are shown as grey dots, and the bars show the back-transformed adjusted mean (95% CI) calculated by an MMRM using square-root-transformed data. All analyses were performed with available data from participants in the IGP-T (N = 141) and including only subgroups of > 10 participants. * $P < 0.05$.

AID, participants with autoimmune disease receiving anti-CD20 therapy; CI, confidence interval; GMT, geometric mean titre; HD, participants on chronic haemodialysis/peritoneal dialysis; ICS, intracellular cytokine staining; IGP-T, T-cell immunogenicity population; KTx, kidney transplant recipients; MMRM, mixed-effects model for repeated measures; PAD, participants with primary antibody deficiency; PWH, people with HIV; RBD, receptor-binding domain.

Figure 6 | Safety of PHH-1V booster vaccination.

Summary of cumulative solicited local and systemic events by maximum intensity from Day 0 to Day 7 after PHH-1V vaccination in the ITT population (N = 238). Percentages of participants are shown by intensity grade and immunocompromising condition group. Numbers inside the bars indicate the percentage of participants within each grade. If a participant experienced more than one event, they were counted once, based on the most severe or most related event.

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 with HIV.

Figure 7 | SARS-CoV-2 infections.

(a) Cumulative incidence of confirmed SARS-CoV-2 infections after PHH-1V booster vaccination (IGP population; N = 221) by immunocompromising condition and overall, shown in black. Each symbol represents a confirmed SARS-CoV-2 case. **(b)** Absolute number of SARS-CoV-2 infections after PHH-1V booster vaccination (IGP population; N = 221) by immunocompromising condition and overall, shown in black, according to calendar month. The number of active participants at risk is shown below each graph.

AID, participants with autoimmune disease receiving anti-CD20 therapy; HD, participants on chronic haemodialysis/peritoneal dialysis; IGP, immunogenicity population; KTx, kidney transplant recipients; mITT, modified intention-to-treat; PAD, participants with primary antibody deficiency; PWH, people with HIV.

832 **Table 1 | Main demographic and clinical characteristics of participants at Day 0.**

	PWH (n = 61)	KTx (n = 37)	HD (n = 59)	PAD (n = 24)	AID (n = 57)	ITT, overall (N = 238)
Age, years,	55	55	71	55	50	56
median (range)	(26–75)	(21–74)	(26–90)	(27–86)	(25–78)	(21–90)
≥ 65 years, n (%)	5 (8)	8 (22)	35 (59)	8 (33)	6 (11)	62 (26)
Male, n (%)	48 (79)	30 (81)	44 (75)	13 (54)	17 (30)	152 (64)
Medical comorbidities						
Hypertension, n (%)	21 (34)	26 (70)	34 (58)	7 (29)	8 (14)	96 (40)
Diabetes mellitus, n (%)	6 (10)	14 (38)	24 (41)	2 (8)	4 (7)	50 (21)
Chronic obstructive pulmonary disease, n (%)	0 (0)	1 (3)	11 (19)	4 (17)	1 (2)	17 (7)
Cardiac disorders, n (%)	3 (5)	9 (24)	21 (36)	2 (8)	1 (2)	36 (15)
Prior SARS-CoV-2 infection*, n (%)	34 (56)	17 (46)	30 (51)	19 (79)	27 (47)	127 (53)
Laboratory parameters at baseline						
IgG, g/L	11.37	9.01	9.42	8.58	8.90	9.80
median (range)	(3.58–23.71)	(3.60–13.44)	(3.12–24.74)	(1.90–16.88)	(4.31–28.02)	(1.90–28.02)
IgA, g/L	2.49	1.87	2.44	0.16	2.16	2.08
median (range)	(0.39–8.86)	(0.11–5.27)	(0.47–13.63)	(0.05–2.18)	(0.48–4.73)	(0.05–13.63)
IgM, g/L	0.79	0.83	0.72	0.25	0.49	0.69
median (range)	(0.14–24.90)	(0.21–2.01)	(0.21–3.52)	(0.02–3.49)	(0.10–2.39)	(0.02–24.90)
CD19⁺ cells/mm³	140	60	70	60	0	60
median (range)	(10–450)	(10–190)	(0–610)	(0–840)	(0–30)	(0–840)
CD3⁺ cells/mm³	1,020	880	960	1350	1170	1,040
median (range)	(220–2,410)	(180–1,740)	(370–3,050)	(130–2,690)	(330–2,480)	(130–3,050)
CD4⁺ cells/mm³	240	410	570	630	750	490
median (range)	(30–600) [#]	(40–890)	(170–1,260)	(80–1,470)	(240–1,950)	(30–1,950)

833 *Based on a history of a positive RAT/PCR test for SARS-CoV-2 or a positive SARS-CoV-2 N-
834 specific serology result at baseline.

835 [#]Value corresponding to the Day 0 assessment. PWH were not excluded if their CD4⁺ T-cell count
836 was > 400 cells/mm³, provided that a CD4⁺ T-cell count of < 400 cells/mm³ had been confirmed
837 within the preceding 6 months before entering the study, as per protocol-defined inclusion criteria.

838 PAD group: 18 (75%) participants had common variable immunodeficiency disorders; two
839 (8.3%) had unclassified antibody deficiency, and four (16.7%) were classified as “Other”. Three
840 (12.5%) participants had IgA/IgG deficiency; 9 (37.5%) had IgA/IgG/IgM deficiency; 3 (12.5%)
841 had IgA/IgM deficiency; and 9 (37.5%) had IgG deficiency. AID: 31 (54%) participants had
842 multiple sclerosis; 8 (14%) had systemic lupus erythematosus/Sjögren’s syndrome; 7 (12%) had
843 rheumatoid arthritis; 3 (5%) had vasculitis; 2 (3.5%) had myositis; and 6 (11%) were classified
844 as “Other”.

845 AID, participants with autoimmune disease receiving anti-CD20 therapy; HD, participants on chronic
846 haemodialysis/peritoneal dialysis; ITT, intention-to-treat population; KTx, kidney transplant recipients;
847 PAD, participants with primary antibody deficiency; PWH, people with HIV; RAT, rapid antigen test.

848 **Table 2 | Analysis of neutralising and binding antibodies at Day 0 and Day 14 against**
849 **Omicron BA.1/BA.2.**

	PWH (n = 59)	KTx (n = 35)	HD (n = 55)	PAD (n = 24)	AID (n = 55)
Neutralising antibodies against Omicron BA.1 ^a					
n (%)	59 (100)	35 (100)	55 (100)	24 (100)	55 (100)
Baseline GMT	1,766	606	632	175	39
(95% CI)	(1,186; 2,632)	(335; 1,095)	(398; 1,004)	(97; 315)	(27; 57)
Day 14 GMT	7,926	1,924	4,910	527	46
(95% CI)	(5,320; 11,809)	(1,065; 3,477)	(3,091; 7,800)	(293; 947)	(31; 66)
GMFR	4.49	3.18	7.77	3.01	1.16
(95% CI)	(3.49; 5.78)	(1.94; 5.19)	(5.21; 11.57)	(1.72; 5.27)	(0.94; 1.44)
Binding antibodies					
n (%)	59 (100)	35 (100)	55 (100)	24 (100)	24 (100)
Baseline GMT	5,322	1,375	7,118	2,063	12
(95% CI)	(3,565; 7,946)	(516; 3,662)	(4,733; 10,706)	(1,229; 3,464)	(5; 29)
Day 14 GMT	38,322	5,012	40,485	4,754	15
(95% CI)	(25,667; 57,217)	(1,881; 13,351)	(26,918; 60,889)	(2,832; 7,983)	(6; 36)
GMFR	7.20	3.65	5.69	2.30	1.23
(95% CI)	(5.58; 9.29)	(2.30; 5.77)	(3.92; 8.26)	(1.56; 3.41)	(0.98; 1.55)
GMFR Fold Change ≥ 2-fold, n (%)	55 (93.20)	19 (54.30)	41 (74.50)	11 (45.80)	6 (10.90)
(95% CI) ^b	(84; 98)	(37; 71)	(61; 85)	(26; 67)	(4; 22)
GMFR Fold Change ≥ 4-fold, n (%)	40 (67.80)	12 (34.30)	34 (61.80)	5 (20.80)	4 (7.30)
(95% CI) ^b	(54; 79)	(19; 52)	(48; 75)	(7; 42)	(2; 18)

850 ^aOmicron BA.2 for the PWH group, measured by VNA.

851 ^bExact CI for the proportions by Clopper-Pearson method.

852 All analyses were performed in the mITT population (N = 228). An MMRM model was used to
853 assess the endpoint on log₁₀-scale for each underlying disease group. In each model, the age group
854 (≥ 18 to < 65 years; ≥ 65 years), visit number (Baseline, Day 14, Day 91, Day 182, and Day 365),
855 Prior-CoV-2 (yes/no), and Prior/No Prior-CoV-2 subgroup-by-visit interaction were included as
856 fixed effects. A compound symmetry covariance matrix was used to model the within-participant
857 error and the Kenward-Roger approximation was employed to estimate the degrees of freedom.
858 The GMT for treatment mean is estimated using LS Mean from the fitted model on the log₁₀
859 scale and back-transformed. Raw data < 20 have been imputed as 20 for analysis. We imputed
860 any zero values to 10 (half the LLOQ) for analysis. Transformations were made on the imputed
861 data.

862 *AID, participants with autoimmune disease receiving anti-CD20 therapy; CI, confidence interval; GMFR,*
863 *geometric mean fold rise; GMT, geometric mean titre; HD, participants on chronic*
864 *haemodialysis/peritoneal dialysis; KTx, kidney transplant recipients; mITT, modified intention-to-treat*
865 *population; PAD, participants with primary antibody deficiency; PWH, people with HIV; VNA, virus*
866 *neutralisation assay.*

Author Contributions

S.B., J.B., J.G.-P., and B.M. conceived and designed the study. S.B., A.S., A.C., J.M., and M.T. contributed to the study design in further study amendments. S.B., S.S., A. H.-G., A.S., M.T., L.B., C. M.-M., A.C., J.M., A.V., and B.M. contributed to clinical development of the study. B.T., D.R., E.P., E. Aparicio, R.B., N.P., J.B., and N.I.-U. conducted all neutralisation analyses. E. Ainsua, N.P., and J.C. designed and conducted cross-reactivity and IgG subclass determinations. E. Aurrecochea, R. P.-C., I.E., L. B., M.P., C.P. and J.G.-P. conducted all T-cell analyses. Y.A.S. undertook the statistical analysis. J.G.-P. and B.M. contributed to data management and overall study coordination. J.G.-P. and B.M. drafted the manuscript. S.B., B.T., C.B., J.M., J.C., M.P., C.P., N. I.-U., A.V., and J.B. critically revised the manuscript for important intellectual content. All authors reviewed and revised the manuscript and approved the decision to submit for publication.

Funding

This study received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 101046118. The trial was also supported by HIPRA SCIENTIFIC, S.L.U. (HIPRA) and partially funded by the Centre for the Development of Industrial Technology (IDI-20230889), a public organisation under the Spanish Ministry of Science and Innovation.

Data Availability Statement

All data relevant to the study are included in the article or uploaded as supplementary information. The datasets for this study are available upon reasonable request to the corresponding authors and with permission of HIPRA SCIENTIFIC, S.L.U. (HIPRA).

Acknowledgments

Special thanks to all the volunteers who participated in this study. We especially acknowledge all HIPRA study team members (Neus Cantariño Iglesias, Júlia Corominas García, Meritxell Deulofeu Figueras, Laura Ferrer Soler, Anna Granés Coll, Irina Güell Casellas, Jon Insunza Gómez, Laia Madrenas Serra, Maria Teresa Prat Cabañas, Toni Prenafeta Amargós, Maria Glòria Pujol Prat, Lluís Riera Sans, and Èlia Torroella) for their support in the study design, clinical development, data management and analysis, and manuscript review. We thank Veristat, which contributed to eCRF development, data management, and database programming for the study. We thank SmartWorking4U for manuscript preparation, which was funded by HIPRA. During

the preparation of this manuscript, the author(s) used AI tools (including Copilot and ChatGPT) for English language proofreading and editing. The final content was reviewed and edited by the author(s), who take full responsibility for the integrity and accuracy of the publication. We thank the HCB-IDIBAPS Biobank, integrated in the Spanish National Biobanks Network, for the biological human samples and data procurement. Finally, we extend special thanks to the members of the RBDCOV Community Advisory Panel (Jennifer Catherine Camaradou, Maka Gogia, Arda Karapinar, Daniela Rojas Castro, and Siegfried Schwarze) for their contributions to reviewing all study materials and overseeing the clinical development of the trial. IrsiCaixa received funds from the “Centres de Recerca de Catalunya” (CERCA) Programme (2021 SGR 00452). L.B. was supported by grant 2024 FI-1 00407.

Conflicts of Interest

C.B. was a shareholder and co-founder of AELIX Therapeutics and reports consultancy fees from Gilead Sciences, Astrivax and Omniscope. **J.B.** reports consultancy fees from NESAPOR EUROPE and HIPRA and, outside the presented work, institutional grants from MSD, NESAPOR EUROPE, and HIPRA. **N.I.U.** has collaborated with the PharmaMar Foundation as part of a scientific jury and reports institutional funding from Pharma Mar, HIPRA, Amassence, and Smart Vitamins. **B.M.** reports consultancy, advisory and/or speaker fees from AELIX Therapeutics, Gilead Sciences, Janssen, ViiV, and MSD. **J.M.** has received research funding, consultancy fees, and lecture sponsorships from, and has served on advisory boards for, MSD, Gilead Sciences, ViiV Healthcare, and Johnson & Johnson. The remaining authors declare that the research was conducted in the absence of any conflict of interest.

RBDCOV HIPRA-HH-4 study group

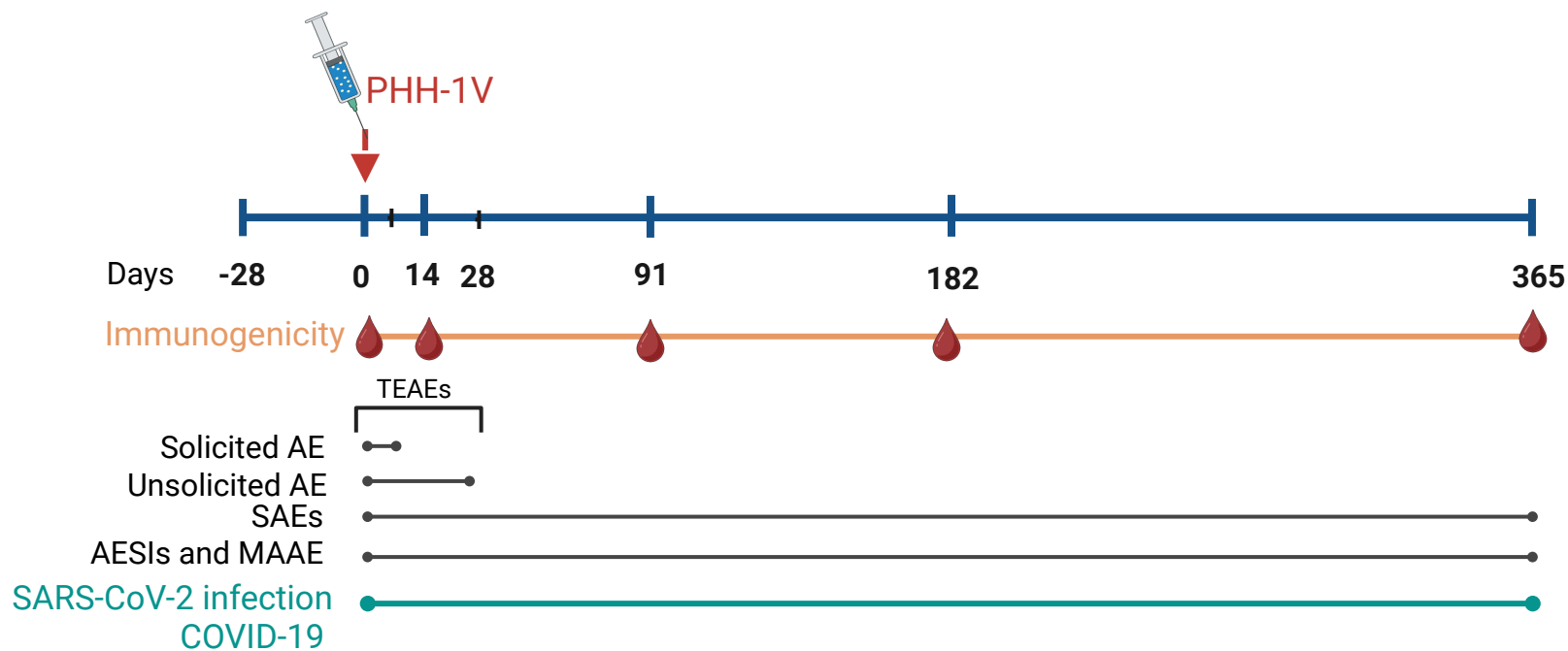
From Fundació Lluïta contra les Infeccions, Badalona, Spain: Yovaninna Alarcón-Soto, Patricia Cobarsí, Cristina Martinez, Anna Martinez-Sagrera, Aroa Nieto, Maria Sarquella, Jordi Puig, and Andrea Vázquez.

From Hospital Universitari Germans Trias i Pujol, Badalona, Spain: Lucía Bailón, Susana Benet, Carme Bracke, Irma Casas, Ivette Casafont-Sole, Maria Esteve, Fredzzia Graterol, José Moltó, Isaac Nuño, Roger Paredes, Maria Lluïsa Pedro-Botet, Sofia Sabato, and Anna Vila-Santandreu.

From IrsiCaixa, Badalona, Spain: Ferran Abancó, Erola Ainsua, Ester Aparicio, Julià Blanco, Rytis Boreika, Christian Brander, Jorge Carrillo, Laia Bernad, Santiago Caverro, Ruth Peña, Raúl Pérez-Caballero, Julia G Prado, Samandhy Cedeño, Eulalia Grau, Nuria Izquierdo-Useros, Silvia

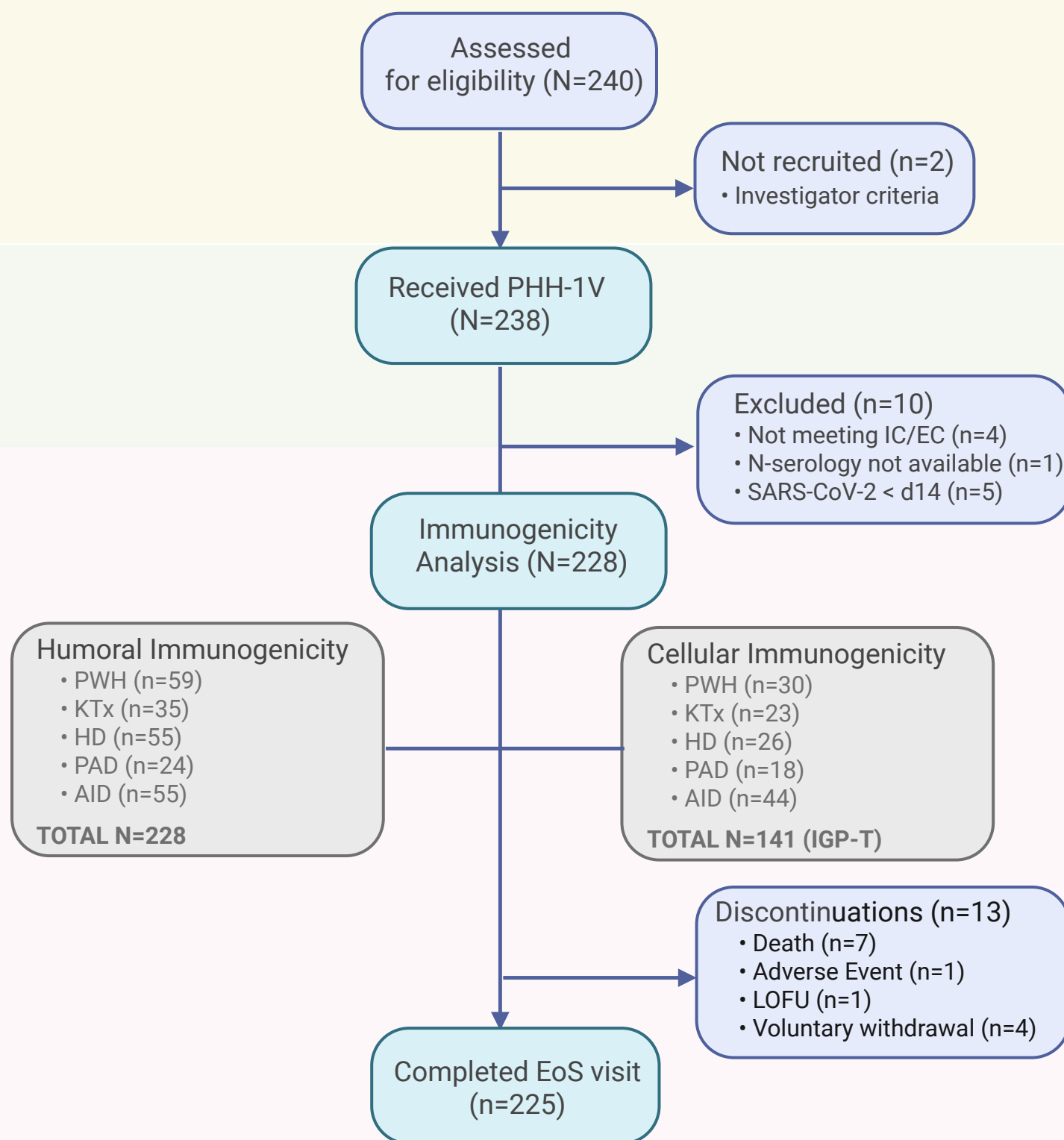
- 930 Marfil, Beatriz Mothe, Núria Pedreño, Cristina Peligero, Tetyana Pidkova, Edwards Pradenas,
931 Dàlia Raïch-Regué, and Benjamin Trinité.
- 932 **From Hospital Clínic-IDIBAPS, Barcelona, Spain:** Elena Aurrecochea, Ignasi Esteban,
933 Fauno Guazina, Sara Messeguer, Antonio Moreno, Jocelyn Nava, Montse Plana, Martha Plazas,
934 Roc Pomarol, Montse Sola, and Alex Soriano.
- 935 **From Hospital Universitari de Girona Doctor Josep Trueta-IDIBGI, Girona, Spain:** Antoni
936 Castro, Abelardo Hurtado Ganoza, Ines Jou Segovia, Cristina Martinez, Anna Oller-Perez, and
937 Xavier Salgado Serrano.
- 938 **From HIPRA, Amer, Girona, Spain:** Alexandra Moros Sanz, Anna Granés Coll, Anna Moya,
939 Èlia Torroella, Irina Güell Casellas, Javiera González, Jon Insunza Gómez, Júlia Corominas
940 García, Laura Ferrer Soler, Lluís Riera Sans, Maria Glòria Pujol Prat, Maria Teresa Prat Cabañas,
941 Meritxell Deulofeu Figueras, Neus Cantariño Iglesias, and Toni Prenafeta Amargós.
- 942 **From MetPharm Arastirma Gelistirme Saglik Danismanlik, Istanbul, Turkey:** Mete Tan.
- 943 **From Ankara University Medical Faculty Internal Medical Sciences Section, Ankara,**
944 **Turkey:** Alpay Azap.
- 945 **From Hacettepe University Medical Faculty Internal Medical Sciences Section, Ankara,**
946 **Turkey:** Serhat Ünal, Gulcin Telli Dizman.
- 947 **From Koc University Medical Faculty Internal Medical Sciences Section, Istanbul, Turkey:**
948 **Önder Ergönül.**
- 949 **From EATG, Brussels, Belgium:** Giorgio Barbareschi.

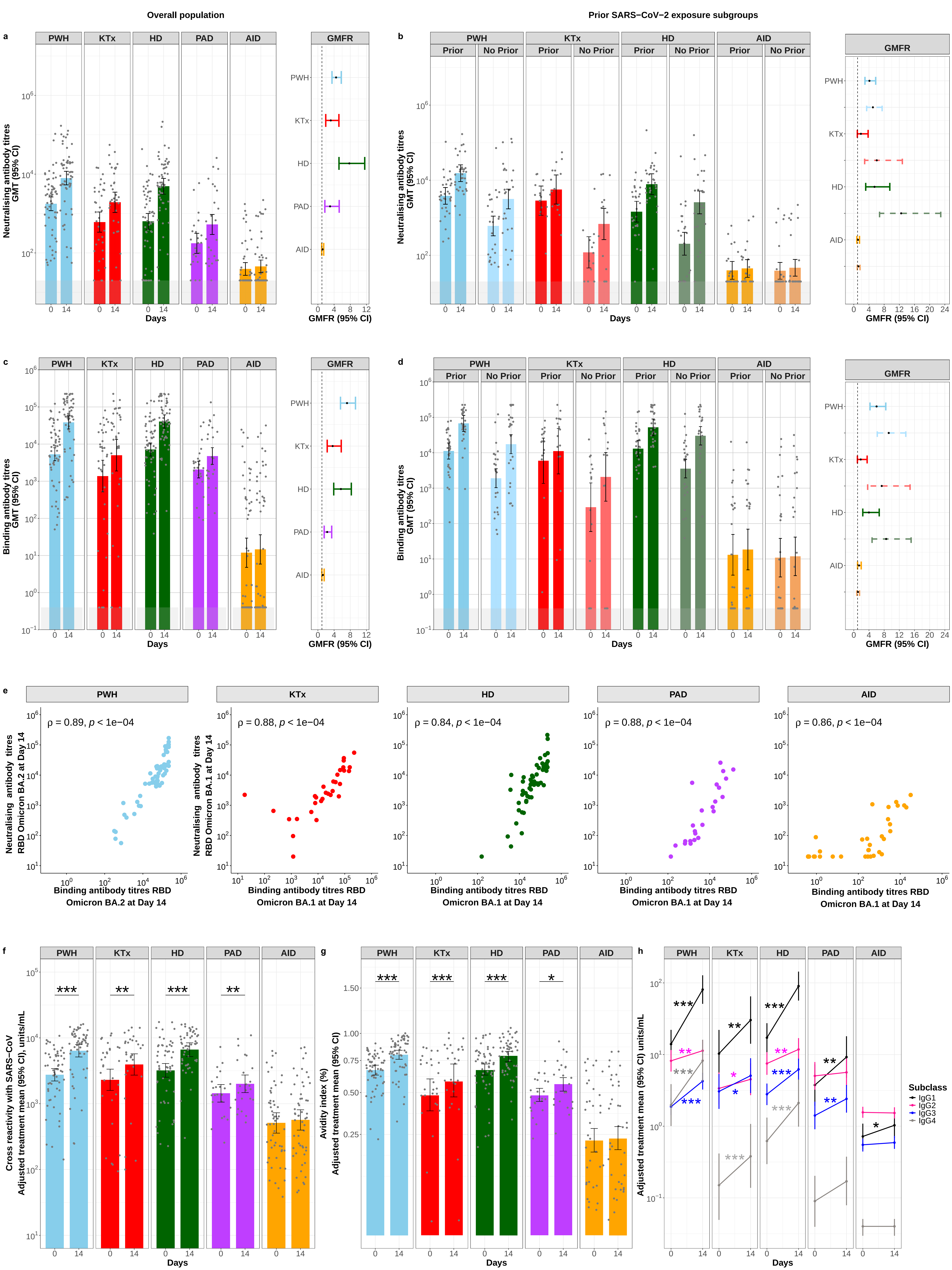
a

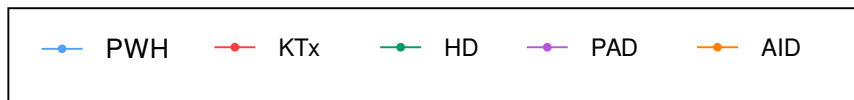


b

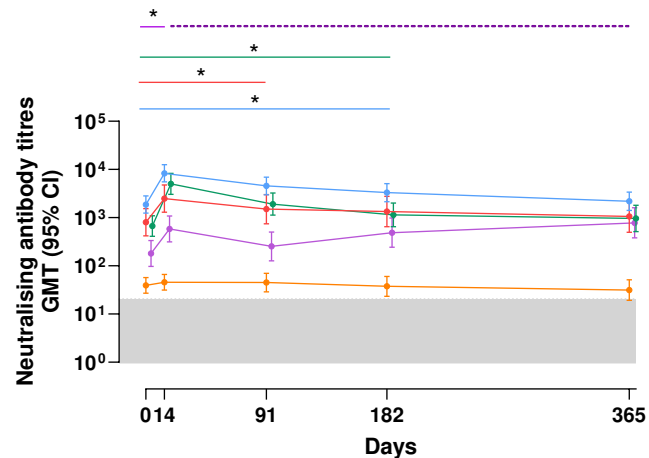
Enrolment

ITT and SP
population
Safety analysismITT population
Immunogenicity Analysis





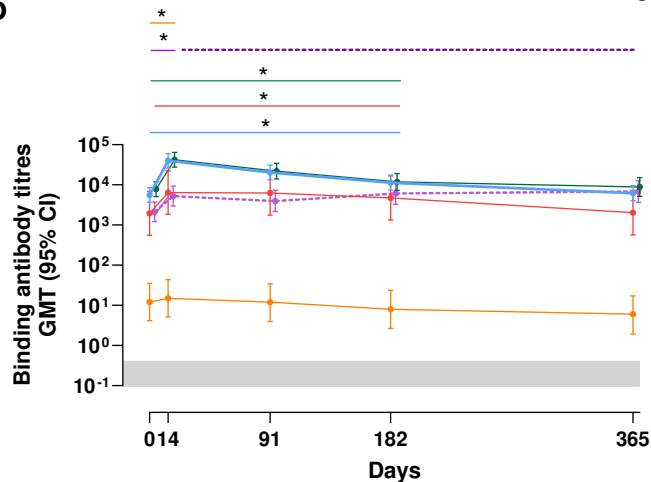
a



Number of participants at each day

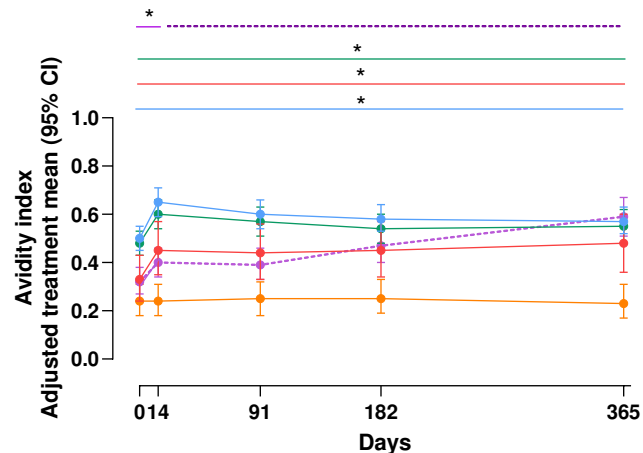
PWH	59	59	53	48	46
KTx	35	35	28	25	18
HD	55	55	44	34	24
PAD	24	24	18	17	16
AID	24	24	18	17	16

b

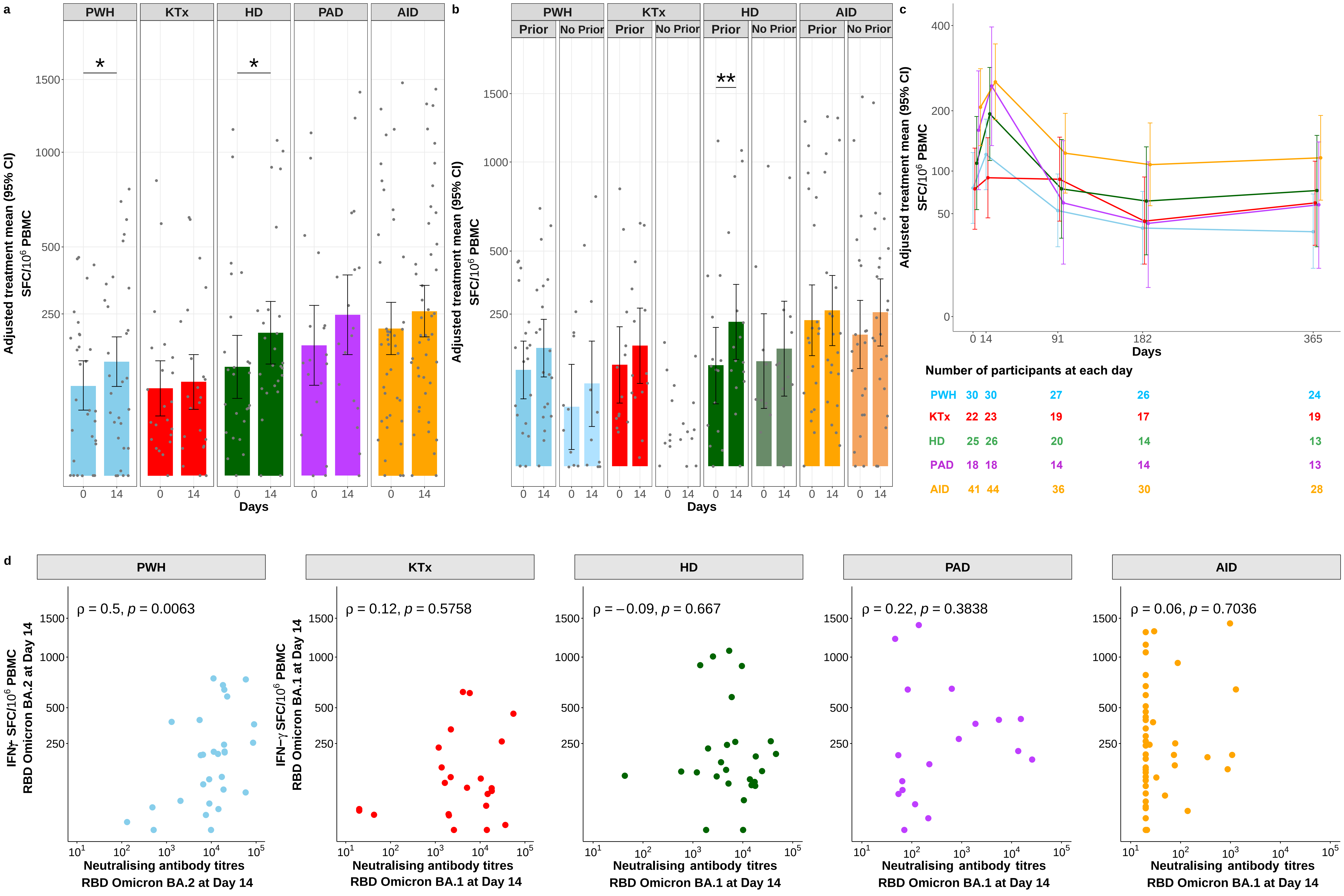


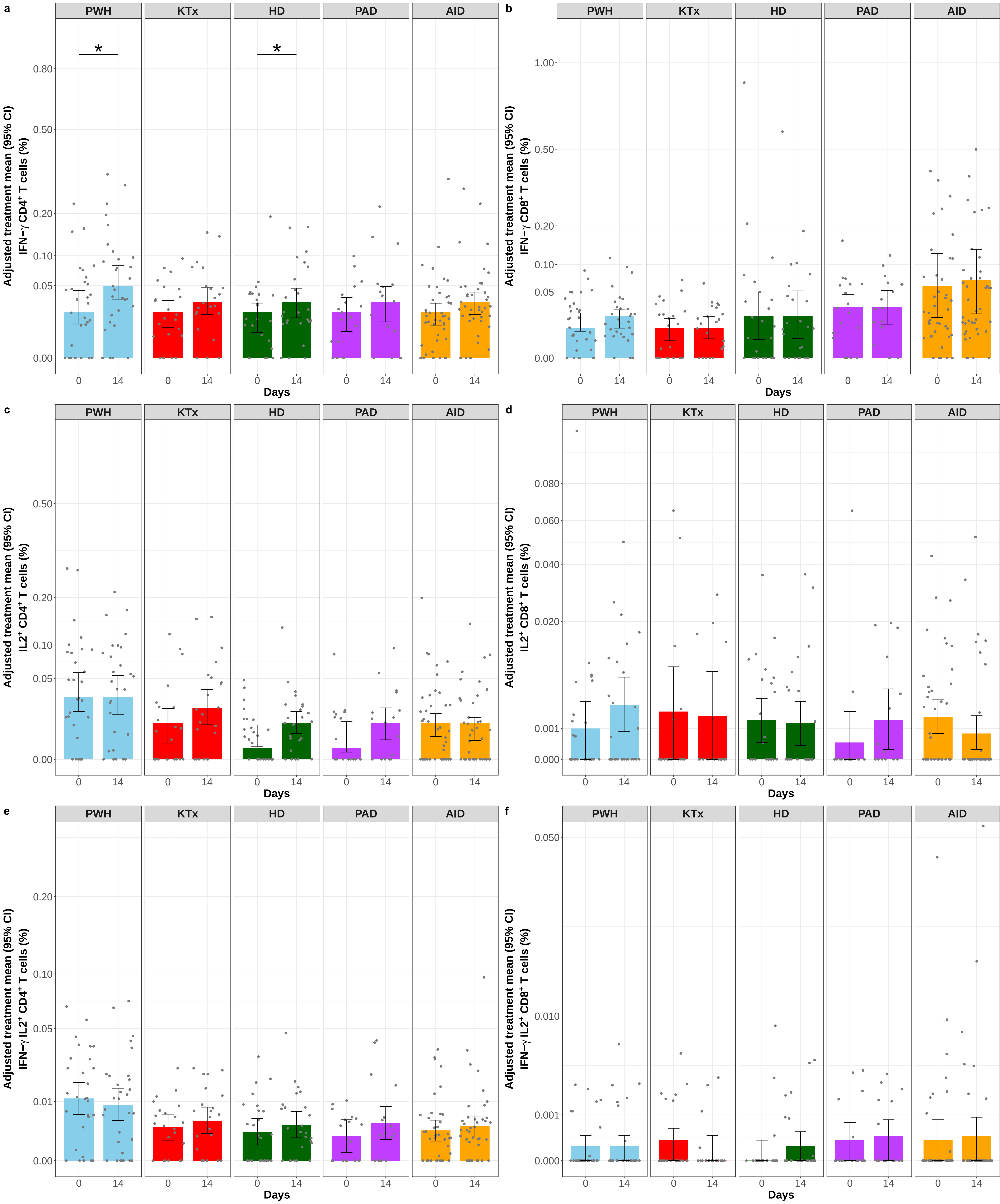
PWH	59	59	53	48	46
KTx	35	35	28	25	18
HD	55	55	44	34	24
PAD	24	24	18	17	16
AID	24	24	18	17	16

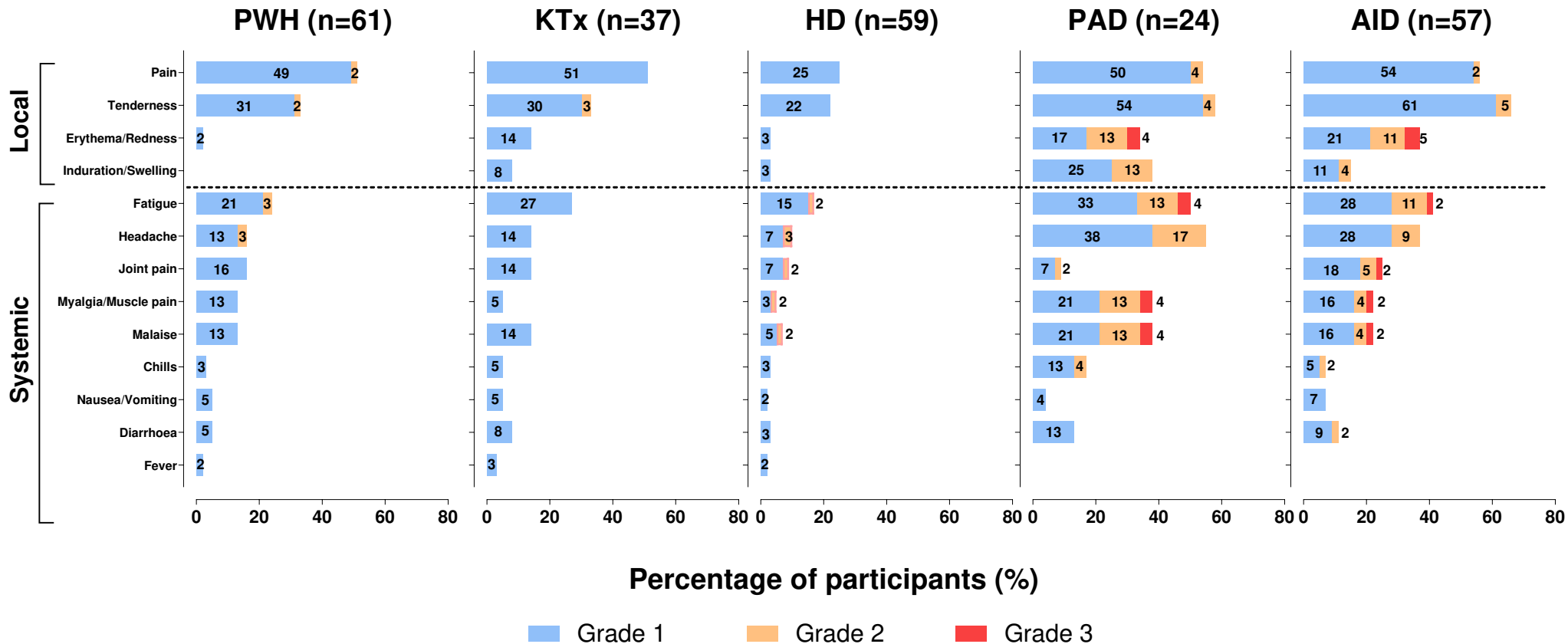
c



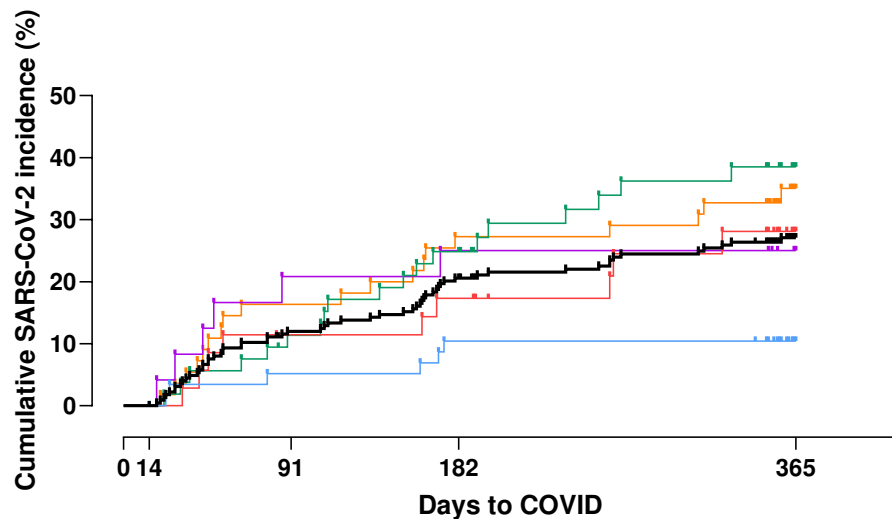
PWH	59	59	53	48	46
KTx	27	28	23	20	18
HD	53	53	42	32	24
PAD	24	24	18	17	16
AID	24	24	18	17	16







a



Participants at risk

Day	0	14	91	182	365
Overall	221	221	186	168	152
PWH	59	59	54	50	50
KTx	35	35	30	28	20
HD	55	55	46	39	27
PAD	24	24	19	18	18
AID	55	55	44	40	37

— Overall

— PWH

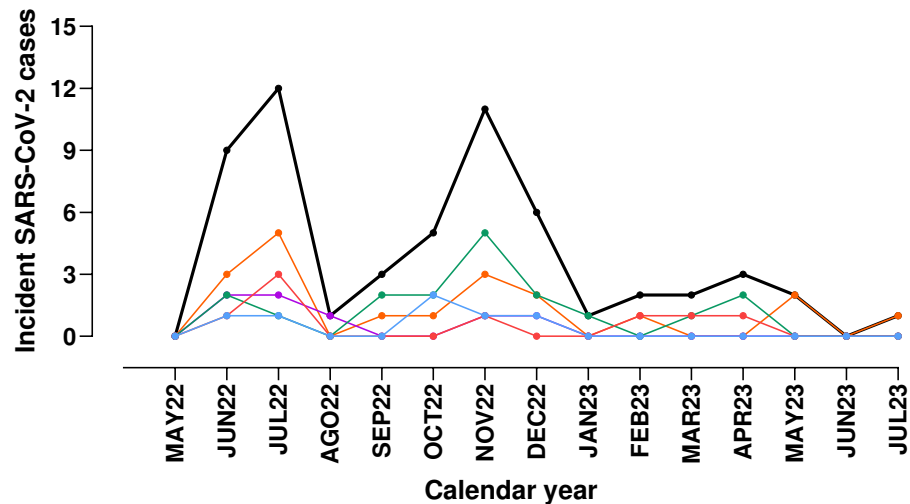
— KTx

— HD

— PAD

— AID

b



Participants at risk (at the end of the month)

Overall	97	213	216	212	211	211	187	161
PWH	26	56	55	51	50	50	26	0
KTx	19	26	25	23	23	25	13	5
HD	7	49	46	36	30	29	27	2
PAD	20	20	19	18	17	17	3	0
AID	25	47	46	42	40	39	29	0

Supplementary Files

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

- [BenetSupplDescription.pdf](#)
- [SupplFigureS5.pdf](#)
- [SupplFigureS6.pdf](#)
- [BenetSuppl.pdf](#)
- [BenetSupplData2.pdf](#)
- [BenetSupplData1.pdf](#)
- [SupplFigureS2.pdf](#)
- [SupplFigureS4.pdf](#)
- [SupplFigureS1.pdf](#)
- [SupplFigureS3.pdf](#)