Longitudinal immunogenicity cohort study of SARS-CoV-2 mRNA vaccines across individuals with different immunocompromising conditions: heterogeneity in the immune response and crucial role of Omicron-adapted booster doses

Annalisa Ciabattini,[a,*] Elena Pettini,[a,j] Fabio Fiorino,[a,b,j] Jacopo Polvere,[a,j] Simone Lucchesi,[a] Chiara Coppola,[a] Simone Costagli,[a] Gabiria Pastore,[a] Anna Sicuranza,[c] Monica Tozzi,[d] Arianna Lippi,[e,f] Francesca Panza,[e,f] Monica Bocchia,[c] Alessandro Bucalossi,[d] Guido Garosi,[g] David Bennett,[h] Sonia Bernazzali,[i] Massimiliano Fabbiani,[e,f] Francesca Montagnani,[e,f] and Donata Medaglini[a]

[a]Laboratory of Molecular Microbiology and Biotechnology, Department of Medical Biotechnologies, University of Siena, Siena, Italy
[b]Department of Medicine and Surgery, LUM University "Giuseppe Degennaro", Bari, Italy
[c]Haematology Unit, Department of Medical Science, Surgery and Neuroscience, University Hospital of Siena, Siena, Italy
[d]Cellular Therapy Unit, Department of Innovation, Experimentation, Clinical and Translational Research, University Hospital of Siena, Siena, Italy
[e]Department of Medical Sciences, Infectious and Tropical Diseases Unit, University Hospital of Siena, Siena, Italy
[f]Department of Medical Biotechnologies, University of Siena, Siena, Italy
[g]Department of Medical Science, Nephrology, Dialysis and Transplantation Unit, University Hospital of Siena, Italy
[h]Respiratory Disease and Lung Transplant Unit, University of Siena, University Hospital of Siena, Italy
[i]Department of Cardiac Surgery, University of Siena, Siena, Italy

eBioMedicine
2025;113: 105577
Published Online xxx
https://doi.org/10.1016/j.ebiom.2025.105577

Summary

Background Individuals with primary and secondary immunodeficiencies, being more susceptible to infections, are a priority for vaccination. Here, we determined and compared in a longitudinal study the immune response elicited by SARS-CoV-2 vaccination across different groups of individuals who are immunocompromised.

Methods In the PatoVac_COV longitudinal prospective single-centre study, the spike-specific B cell and antibody responses to SARS-CoV-2 mRNA vaccination were compared across 5 different groups of individuals with haematological malignancies, hematopoietic stem cell (HCT) or solid organ transplantation (SOT), undergoing haemodialysis, and people living with HIV (PLWH), for a total of 585 participants. Data from participants who were immunocompromised were compared to a group of 123 participants who were immunocompetent. Blood samples were collected before and after each vaccine administration, up to 2 years.

Findings A different immune responsiveness was observed after the first two vaccine doses, with haematological, haemodialysis, and SOT participants showing reduced responsiveness compared to HCT and PLWH, and relative to the comparison group. Spike-specific B cell response was both slower and lower in all groups except in PLWH when compared to participants who were immunocompetent. However, the first booster dose enhanced both the B and the antibody responses in all groups, that persisted up to 2 years after the first vaccine administration. The administration of Omicron-adapted booster vaccines promoted a primary BA.2 RBD-specific B cell response, especially in participants who were immunocompromised. Despite repeated vaccinations, a subset of persistent low-responders, especially among SOT, was identified.

Interpretation Our study highlights the heterogeneous immune response across individuals with different pathologies, the pivotal role of the first booster dose, the primary activation of Omicron-specific B cells elicited by updated variant-adapted vaccines and the persistence of low-responders despite multiple vaccine administrations. These aspects have a clinical relevance for planning vaccination schedules tailored for individuals with different immunocompromising conditions.

*Corresponding author. Department of Medical Biotechnologies, University of Siena, Siena, Italy.
E-mail address: annalisa.ciabattini@unisi.it (A. Ciabattini).
[j]Shared second authors.

Funding This work was supported by funds from the Department of Medical Biotechnologies of the University of Siena, and from EU within the NextGenerationEU-MUR PNRR Tuscany Health Ecosystem (Project no ECS00000017-THE).

Keywords: Immunocompromised; Vaccine immunogenicity; mRNA vaccines; Memory B cells; SARS-CoV-2

Research in context

Evidence before this study

After four years from the beginning of the worldwide vaccination campaign against SARS-CoV-2, a consistent amount of immunogenicity and safety data have been obtained in individuals who are immunocompetent, while much less has been provided respect to individuals who are immunocompromised, with only few clinical trials ongoing. In August 2023, the group of Hans-Gustaf Ljunggren, published here a longitudinal analysis upon mRNA vaccination across different immunocompromised groups, investigating the vaccine antibody response.

Added value of this study

The present prospective cohort single-center study is a longitudinal analysis of the immunogenicity of the mRNA vaccination spanning up to two years post administration of the first vaccine dose, across 5 different groups of individuals who are immunocompromised, comprising a total cohort of 585 subjects. Unlike data obtained in systematic reviews of literature and meta-analyses, our study is founded upon participants recruited from a single clinical site, adhering to the same experimental protocol, and tested through immunological assays conducted within the same laboratory setting. This approach has allowed a comparative longitudinal analysis across the different pathologies, eliminating the limitations associated with studies variability. Since the generation of immunological memory is a requisite of long-term protection in acute infection, the study aimed at comparing not only the serological response but also the induction and the phenotype of spike-specific memory B cells across the different groups. Considering the long period covered by the present study, we have taken into consideration the effects of the Omicron-adapted vaccines, administered as the fourth and fifth doses, as well as natural SARS-CoV-2 infection.

Implications of all the available evidence

Individuals who are immunocompromised represent a critical heterogenous category of people particularly susceptible to infection and generally less responsive to vaccination. The SARS-CoV-2 pandemic has further highlighted the infection susceptibility of these individuals, and the necessity of protecting them through specifically designed vaccination approaches. The in depth understanding of their immune responsiveness to SARS-CoV-2 vaccination, including the impact of booster doses adapted to the new circulating viral variants, can be instrumental to refine and rationally design vaccination schedules against future epidemic/pandemic pathogens. We highlighted the different reaction to vaccination among individuals affected by different pathologies, that are often considered together as a single category of people. Indeed, while some pathologies were much impacting in the immune responsiveness, others, such as hematological stem cell transplantation and HIV infection, had a less impact, and participants developed an immune response more similar to individuals who are immunocompetent, since the first vaccine administration. Updated variant-adapted booster vaccines should be recommended for all individuals who are immunocompromised, in order to promote a B cell response specific for the circulating viral variants. Nevertheless, individuals permanently remaining low responders, as observed among participants who were undergoing haemodialysis and solid organ transplantation, need to be specifically considered for alternative prophylactic approaches, such as monoclonal antibodies.

Introduction

The SARS-CoV-2 pandemic has offered a unique opportunity to rapidly generate a vast amount of data on the immunogenicity, safety, and efficacy of various vaccines, including those based on the novel mRNA-based vaccine platform. Additionally, it has provided the chance to analyse immune responses in individuals often excluded from clinical trials, such as those who are immunocompromised. Now, four years later, it is crucial to analyse and capitalise on these highly relevant data, seeking useful insights to prepare for potential future pandemics or epidemics.

Vulnerable individuals comprise an heterogenous group of moderately or severely immunocompromised individuals, including those with haematological malignancies, solid cancer, recipients of hematopoietic stem cell or solid organ transplants, chronic infections, autoimmune diseases, or those affected by diseases requiring immunosuppressive therapies. Individuals who are immunocompromised are more susceptible to infections and often exhibit a reduced responsiveness to vaccination,[1,2] due to immunological disorders and pharmacological treatments. We and others have observed a different immune responsiveness to the SARS-CoV-2 vaccination among different groups of individuals who are immunocompromised,[3–8] strengthening the idea that vaccination schedules specifically tailored for individuals with different pathological conditions should be developed. With the emergence of the Omicron variant of concern in November 2021, vaccine-induced protection was further reduced. Since September 2022, bivalent Omicron-adapted vaccine booster doses have been provided, especially to individuals who are immunocompromised, however immunogenicity data remain limited.[9,10]

Even though the induction, persistence, and neutralization activity of antibodies have been initially considered as the main parameter to be analysed in the context of SARS-CoV-2 vaccination, it is well recognized the fundamental role of the immunological memory and the importance of the persistence of spike-specific memory B[11,12] and T cell responses.[13] The T immune response has been characterized in both individuals who are immunocompetent and immunocompromised,[8,14] showing to be involved in the generation of neutralizing humoral response, and contributing to protection, particularly in individuals with impaired B immune responses.[15] On the other hand, the memory B immune response is crucial for providing long-term protective immunity upon re-exposure to the pathogen, even after an extended period. As we have recently demonstrated, B cell response is affected by impaired immune system functionality, and different individuals who are immunocompromised have shown a lower and delayed spike-specific B cell response,[3,5,16] or a different phenotypic profile.[6] So, it is particularly important to characterize the vaccine-specific B cell response following mRNA-based vaccinations considering its first use in humans.

While several studies have characterized the immune response, particularly the spike-specific antibody response, in single immunocompromising pathologies, few approaches have transversally analysed and compared the immune responsiveness to vaccination across different groups of individuals who are immunocompromised.[15,17–19]

This study aims to longitudinally assess the antibody and B cell responses across various groups of participants who were either immunocompromised or immunocompetent, within the context of the single-center prospective PatoVac_COV clinical study conducted at Siena University Hospital, Italy, from the beginning of the SARS-CoV-2 vaccination campaign. A total of 585 participants who were immunocompromised, including individuals with haematological malignancies, undergoing haemodialysis, hematopoietic stem cell or solid organ transplantation, and people living with HIV, along with a group of participants who were immunocompetent, were enrolled. The same experimental protocol and immunological assays were applied to samples from the different groups thus allowing a longitudinal and transversal analysis across the different pathologies. While interim results among single groups have been partially published,[3–6,16] this is the first comparative testing across the five groups that covers a period up to 2 years after the start of SARS-CoV-2 vaccination.

Immunological parameters compared among the different groups include the characterization of the spike-specific B-cell response, in terms of memory B cell subsets and persistence overtime as well as the spike-specific IgG antibody induction, functionality and persistence. Considering the emergence of antigenically diverse viral variants, and the development of vaccine formulations adapted to the Omicron variant, we have differentiated the long-term immune response analysis according to the wild type and Omicron BA.2 variant-reactivity. Furthermore, the groups have been separated between participants reporting or not breakthrough infection, to consider the impact of the natural infection on the humoral and B cellular immune responses.

The longitudinal immunological analysis conducted across the various groups of individuals who are immunocompromised upon vaccination, aimed to address the following aspects: *i)* comparing the antibody response against SARS-CoV-2 after the 2-dose primary vaccination among individuals affected by different immunocompromising pathologies, and *versus* the immunocompetent comparison group; *ii)* investigating the impact of booster doses on antibody and spike-specific B cell responses in individuals affected by different immunocompromising pathologies, and comparing data with those of immunocompetent individuals, 30 and 180 days after the booster administration; *iii)* assessing the persistence of the immune response at 24–30 months from the first immunisation, among the groups of HD, SOT and PLWH *versus* the immunocompetent group, stratifying each group according to the occurrence of breakthrough infections.

Methods

Study population

Individuals affected by different immunocompromising pathologies and those who were immunocompetent were enrolled in two prospective longitudinal clinical studies, named PatoVac_COV and IMMUNO_COV respectively, conducted at the Siena University Hospital, Siena, Italy. The IMMUNO_COV study started in January 2021 recruiting 123 participants without immunocompromising disorders or therapeutic treatments and without significant co-morbidities who were used as immunocompetent comparison group. Immunocompetent participants were recruited by an information campaign among citizens, healthcare workers and university teaching staff. After informed consent, all immunocompetent participants were evaluated by clinical investigators to rule out diseases with a potential impact on immunological response. Participants were recruited at Azienda Ospedaliera Universitaria Senese, the only referral hospital for heart and lung transplants in the Tuscany region (Central Italy). It also serves as the referral hospital for both the general population and other types of studies, including those involving individuals who are immunocompromised, within the province of Siena. The PatoVac_COV study started in March 2021, enrolling participants with hematopoietic stem cell transplantation (HCT, n = 56 allogenic transplants), undergoing haemodialysis (HD, n = 58), solid organ transplantation (SOT total n = 198, heart transplantation n = 143, lung transplantation n = 55), people living with HIV (PLWH, n = 113 all currently on antiviral therapy, and 80% with stable CD4 cells counts >350/mmc and undetectable viral load) and haematological malignancies (HM, n = 160). The latter group included participants affected with acute myeloid leukaemia (AML, n = 17), chronic lymphocytic leukaemia (CLL, n = 30), lymphoma (n = 54), multiple myeloma (MM, n = 21) and primary myelofibrosis (MF, n = 38). Participants were recruited at Azienda Ospedaliera Universitaria Senese, at the Cellular Therapy Unit (HCT), Nephrology, Dialysis and Transplantation Unit (HD), Respiratory Disease and Lung Transplant Unit and Cardiac Surgery Unit (SOT), Infectious and Tropical Diseases Units (PLWH) and Haematology Unit (HM). For the PatoVac_COV study inclusion criteria were at least one of the aforementioned immunocompromising conditions. Inclusion criteria for both PatoVac_COV and IMMUNO_COV studies were age ≥18 years and adherence to the SARS-CoV2 vaccination campaign. Exclusion criteria for both studies were pregnancy, withdrawal of consent or refusal to participate, clinical problems for collecting additional blood samples beyond the amount required for routine care. For the IMMUNO_COV study, an exclusion criterion was to be affected by any immunocompromising condition (congenital, acquired, or drug-related).

Vaccine administration was carried out according to the national program by general practitioners or by vaccination centers, specifically dedicated to SARS-CoV-2 vaccination according to the indications of the Italian Ministry of Health, with different schedules (three to five doses) according to age, health conditions and occupation. Participants received the first two doses of BNT162b2 (Comirnaty®; Pfizer-BioNTech), or mRNA-1273 (Spikevax®, Moderna) vaccines, administered 21 and 28 days apart, respectively, according to the national guidelines (2-dose primary vaccination), and boosted (third to fifth doses) with BNT162b2 or mRNA-1273 vaccines 5–7 months (third dose), 12–18 months (fourth dose) and 19–21 months (fifth dose) from the first vaccine dose. All booster doses administered until October 2022 were performed with the Wuhan original monovalent vaccines according to the national vaccination program. Boosters administered afterwards may be either the original monovalent formulation, the bivalent one (Original/Omicron BA.1 or Original/Omicron BA.4-5) or the monovalent Omicron XBB.1.5. Data on formulations administered in each group are reported in supplementary tables (Supplementary Tables S1–S6).

SARS-CoV-2 infection in participants who were immunocompetent was detected through passive surveillance. Participants were instructed to complete two surveys to collect data regarding potential infection, the first one in February 2022, and the second in May 2023. Moreover, they were asked to update their infection status each time they were called for blood collection. SARS-CoV-2 infection was clinically identified based on the presence of symptoms or confirmed close contact with individuals who were infected. Diagnosis was established via antigenic or molecular testing, conducted on nasopharyngeal swabs, either self-administered or collected by healthcare professionals. Participants were asked to specify the date of the positive test in the survey. Participants who were immunocompromised updated infection-related information directly with their clinicians each time they were called for blood collection, including the date of the positive antigenic or molecular test, which was similarly conducted on self-administered or healthcare professional-collected nasopharyngeal swabs. Participants reporting SARS-CoV-2 infection from December 2021, were assumed to be infected by Omicron, the prevalent variant in Italy from December 2021.[20]

Ethics

Written informed consent was obtained from the participants according to the Declaration of Helsinki/International Conference on Harmonization Guideline for Good Clinical Practice. The studies were performed in compliance with all relevant ethical regulations and the protocols were approved by local Ethical Committee for Clinical experimentation (CEAVSE; protocol code n.19479, approved on 3rd March 2021 for PatoVac_COV, and n.18869 v1.0 approved on the 21st December 2020 for IMMUNO_COV).

Blood sample collection

Blood samples were collected at the baseline (the same day of the first vaccine dose before vaccine administration), 10–30 days and 4–6 months post the second dose, 30 days and 4–6 months post the third dose, 30 days post the fourth doses, and 24–30 months post the first dose. Not all participants in the groups provided blood samples at every time point of the study, as detailed in Supplementary Figures S1 and S2. Venous blood samples were collected in heparin-coated blood tubes (Vacutainer; BD, NJ, USA). PBMCs were isolated by density-gradient sedimentation, using Ficoll–Paque (Lympho-prep, Meda, Italy). Cells were gently resuspended with warm cell recovery medium [10% DMSO (Thermo Fisher Scientific, MA, USA) and 90% heat inactivated fetal bovine serum (Sigma Aldrich, MO, USA)] and then rapidly transferred to cryovials that were stored in liquid nitrogen. Plasma samples were stored at –80 °C.

Antibody tests

Spike-specific IgG were tested in all plasma samples by ELISA, as previously described.[4] Briefly, microtitre plates were coated with 1 μg/mL SARS-CoV-2 wild type (wt) or BA.2 full spike protein (S1 + S2 ECD, Sino Biological, China), blocked and added with heat-inactivated plasma samples. Anti-human horseradish peroxidase (HRP)-conjugated IgG was added for 1 h and plates were developed with 3,3′,5,5′-Tetramethylbenzidine (TMB; Thermo Fisher Scientific) substrate. The absorbance was measured at 450 nm using a Multiskan FC Microplate Photometer (Thermo Fisher Scientific). Data are reported as antibody end point titres, calculated as the reciprocal of the sample dilution with the OD value double compared to the background.

Inhibition of the binding between ACE2 and RBD (Wuhan-Hu-1 strain and Omicron BA.2 variant) was tested with a SARS-CoV-2 surrogate virus neutralization test (sVNT) kit (cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit, Genscript, Piscataway, NJ, USA) according to the manufacturer protocol, as previously described.[11] Briefly, plasma samples were diluted 1:20 and incubated with HRP-wt RBD or HRP-BA.2 RBD for 30 min, 37 °C. Mixtures were added to ACE2 pre-coated wells and incubated for 15 min, RT. After substrate addition and development for 15 min RT, the absorbance was measured at 450 nm on a Multiskan FC Microplate Photometer (Thermo Fisher Scientific). Inhibition values ≥ 30% were considered as positive results, and values < 30% as negative results, as previously established[21] and indicated by the manufacturer.

Multiparametric flow cytometry

Spike-specific B cells were identified among PBMC by flow cytometry. The biotinylated wt spike, wt RBD and BA.2 RBD antigens were tetramerized with fluorescently labelled streptavidin (SA) as follows: spike S1+S2 ECD-His recombinant biotinylated-protein (Sino Biological) with SA-R-Phycoerythrin (PE), wt RBD recombinant biotinylated-protein (BioLegend) with SA-Allophycocyanin (APC), BA.2 RBD recombinant biotinylated-protein (ACROBiosystems) with SA-BUV737 (BD Biosciences). Two million of PBMCs were incubated with BD human FC block (BD Biosciences) for 10 min at RT, then with fluorescent antigens for 1 h at 4 °C, and subsequently stained for 30 min at 4 °C with the following antibodies: CD3-BV650 (clone SK7); CD20-APC-H7 (clone 2H7), CD27-BV786 (clone M-T271), CD21-FITC (clone B-ly4), CD19-BUV395 (clone SJ25C1), IgM-BV605 (clone G20-127), IgD-BV711 (clone IA6-2), IgG-PE-Cy7 (clone G18-145, all from BD Biosciences), IgA-Vio blue (clone IS11-8E10; Miltenyi Biotec). After staining, cells were labelled with Zombie Aqua™ Fixable Viability Kit (Thermofisher) according to the manufacturer instruction and fixed with BD fixation solution (BD Biosciences). All antibodies were titrated for optimal dilution. About 10^6 cells were acquired and stored for each sample with SO LSRFortessa X20 flow cytometer (BD Biosciences). Data analysis was performed using FlowJo v10 (FlowJo, LLC, Becton Dickinson and Company, USA).

t-SNE

The antigen specific B cell population was gated on live, singlet, $CD3^{-/}$ $CD19^{+}$ RBD wt^{+} or RBD $BA2^{+}$ cells using FlowJo v10 (TreeStar, USA). Ag-specific B cell data were then exported as .fcs files and imported in R environment as flowSet object, that was then compensated with FlowCore package v2.14.2 and logicle transformed.[22] t-SNE (t-Distributed Stochastic Neighbor Embedding)[23] dimensionality reduction was performed with Rtsne package v0.17. Expression values of each marker were normalized as z-scores (mean = 0 and standard deviation = 1). Rtsne function was run setting perplexity = 100, selected as optimal parameter value in a range between 30 and 200. B cells were analysed with manual gating and gated to detect activated memory ($CD27^{+}CD21^{-}$), resting memory ($CD27^{+}CD21^{+}$), atypical/DN2 ($CD27^{-}CD21^{-}$) and DN1 ($CD27^{-}CD21^{+}$) phenotypes, expressing either IgM, IgG or IgA. Labels of activated and resting memory and DN1 and DN2 populations were imported in R environment using GetFlowJoLabels function from FlowSOM package v2.10.0 for a combined visualization into t-SNE map.

Statistical analysis

At the time of the study design no prior information existed regarding the expected serological and cellular immune responses in SARS-CoV-2 vaccinees. Using an alpha level of 0.05 and a beta level of 0.2, we calculated that a minimum of n = 30 samples was necessary to detect differences with a fold change greater than 1.5, assuming a mean/SD ratio greater than 2. Therefore, in the recruitment of the groups, we exceeded this sample size to ensure sufficient statistical power.[24] Sample sizes for each group in each time point were indicated in Supplementary Figures S1 and S2. By visual inspection (Q–Q plots and histograms) it was observed that not all features (antibody titres, antigen specific B cell frequencies and phenotypes) followed a Gaussian distribution, so only non-parametric statistical tests were employed in this study. All tests were two-tailed, as no prior assumptions were made before the analyses.

Numeric variables were reported as medians with interquartile ranges (IQR) as measure of dispersion, and binary variables were reported as counts and percentages. To measure the recall effect of the booster doses on the spike-specific antibody levels a fold change factor was calculated. The factor was expressed as the ratio between the logarithm of the antibody titres measured after and before each vaccine administration. To assess the significancy, the fold increases were converted to ranks at each time point, and the ranks of each group were compared with the aggregated ranks of the other groups using the Mann–Whitney test. Differences in number of participants positive for the SARS-CoV-2 surrogate virus neutralization test were assessed between immunocompromised groups and immunocompetent control group using Fisher's exact test. For the measurement of spike-specific memory B cells, each group of immunocompromised participants was compared with a subset of immunocompetent participants matched for age (a 10-year age range), sex (up to a 20% deviation from the gender ratio) and time of blood collection (30 ± 5 days after vaccine administration). Frequencies of spike-specific MBC in each group *versus* the respective control group were analysed with unpaired Mann–Whitney test without matching single individuals. To evaluate the impact of the vaccination with or without breakthrough infection, we stratified the groups of HD, SOT, PLWH and immunocompetent participants in infected and non-infected individuals. Multiple comparisons of antigen-specific antibodies were assessed using the Mann–Whitney test for comparisons between infected and non-infected groups, and with the Kruskal–Wallis test followed by Dunn's test for assessing statistical differences between groups of participants who were immunocompromised and infected *versus* participants who were immunocompetent and infected, or participants who were immunocompromised and non-infected *versus* participants who were immunocompetent and non-infected. Multiple comparisons of antigen-specific antibodies and B cell phenotypes were assessed using the Mann–Whitney test for comparisons between infected and non-infected groups, while the Kruskal–Wallis test followed by Dunn's test was used for comparisons across different groups. Correlations between antigen-specific B cells and antibody titres were tested using Spearman's method. P-values were adjusted for multiple comparisons using the Benjamini-Hochberg method. Differences were considered significant at $P < 0.05$, unless otherwise stated. All analyses were performed using GraphPad Prism v9 (GraphPad Software, San Diego, CA, USA) or R v4.3.3 environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria).

Role of the funding source

This work was supported by funds from the Department of Medical Biotechnologies of the University of Siena, and from EU within the Next Generation EU-MUR PNRR Tuscany Health Ecosystem (Project no ECS00000017-THE). The funders had no role in study design, data collection, data analyses, interpretation, or writing of report.

Results

Participants

Starting from January 15, 2021, for the IMMUNO_COV study, and March 4, 2021, for the PatoVac_COV study, a total of 708 participants were included in this observational study and followed up for 24/30 months upon the first vaccine administration. The spike-specific antibody and B cell responses elicited by SARS-CoV-2 vaccination were compared across 5 different groups of participants who were immunocompromised enrolled in the PatoVac_COV study, and 123 participants who were immunocompetent (IC) enrolled in the IMMUNO_COV study. Participants who were immunocompromised included those with haematological malignancies (HM = 160, of which 17 with acute myeloid leukaemia [AML], 30 with chronic lymphocytic leukaemia [CLL], 54 with lymphoma, 21 multiple myeloma [MM], and 38 with primary myelofibrosis [MF]), hematopoietic stem cell transplantation (HCT = 56), solid organ transplantation (SOT = 198), undergoing haemodialysis (HD = 58) and people living with HIV (PLWH = 113; Fig. 1a–c). Baseline characteristics for the different groups are presented in Table 1, while detailed description of each group is reported in Supplementary Tables S1–S6.

All participants who were immunocompromised received the 2-dose primary vaccination and the third dose, administered approximately 5–7 months after the initiation of vaccination. However, among participants with haematological malignancies, only those with primary myelofibrosis provided the blood samples after the third dose, and then they withdrew from the study. The fourth dose was administered to 76% of HD, 95% of HCT, 81% of SOT, and to 16% of PLWH, about 7–10 months after the third dose (12–18 months from the first dose). Omicron-adapted vaccines were provided to 12% of SOT and 21% of PLWH. The HCT group withdrew from the study after the fourth dose. The fifth dose, consisting solely of the Omicron-adapted formulations, was administered to 41% of HD, 39% of SOT, and 4% of PLWH approximately 7–9 months after the fourth dose (19–21 months from the first dose).

Blood samples were collected from all participants, 30 and 180 days after both the second and the third doses, and 30 days after the fourth dose. The blood sample at 24–30 months from the starting of vaccination was collected from IC participants and HD, SOT, PLWH participants. The number of participants providing the blood samples for each time point for

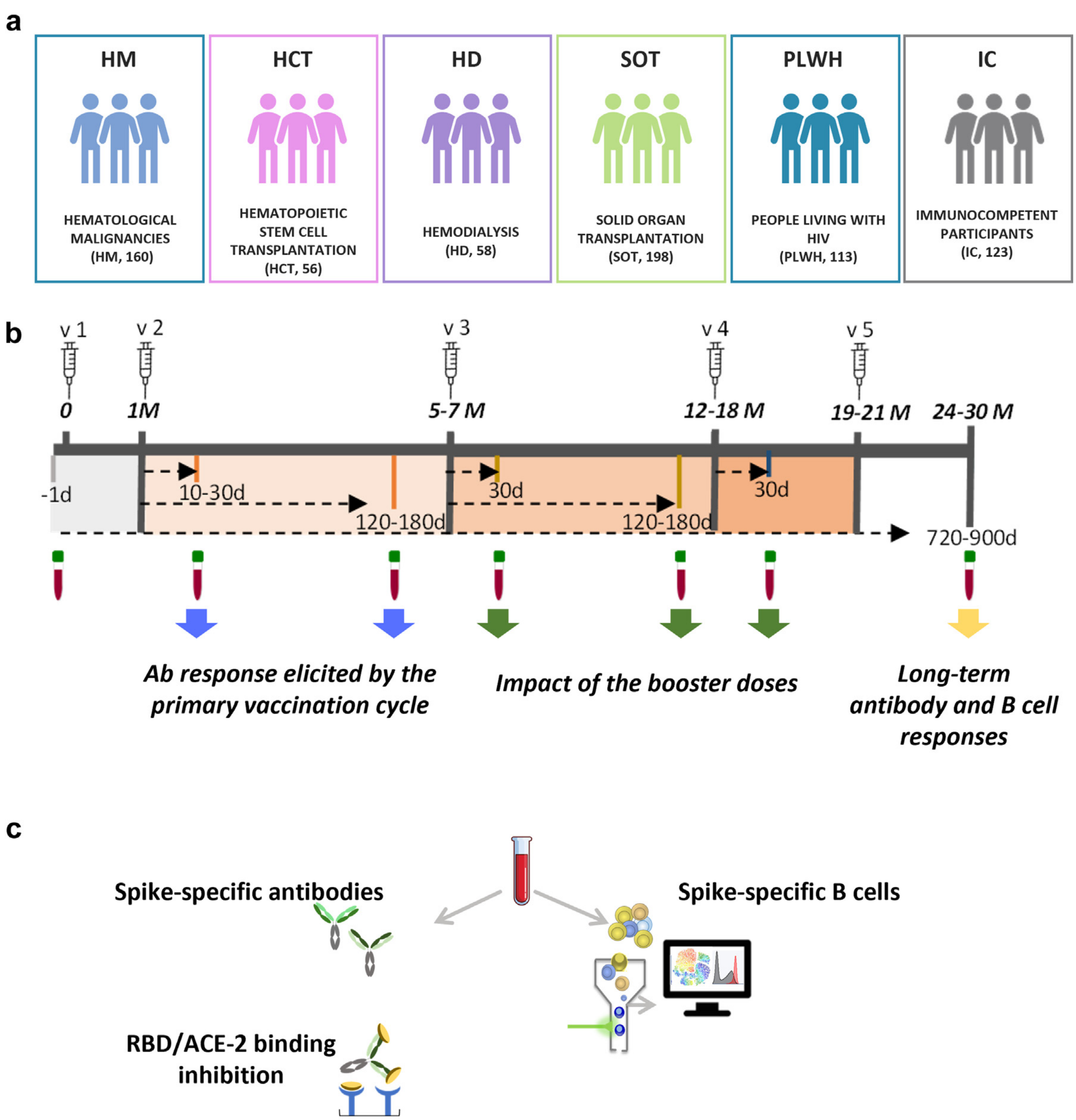

Fig. 1: **Groups of individuals with different immunocompromising pathologies, study design and data analysis**. **a)** A total of 585 participants, belonging to 5 different immunocompromising pathology groups, such as individuals with haematological malignancies (HM, n = 160), hematopoietic stem cell transplantation (HCT, n = 56), undergoing haemodialysis (HD, n = 58), solid organ transplantation (SOT, n = 198) and people living with HIV (PLWH, n = 113), along with a group of individuals who are immunocompetent (IC, n = 123) were enrolled in the study. **b)** mRNA vaccination was performed at day 0 (v1), 21–28 days (v2), 5–7 months (v3), 12–18 months (v4) and 19–21 months (v5) from the first vaccine dose. Blood samples were collected at the baseline, 10–30 days and 4–6 months post the second dose, 30 days and 4–6 months post the third dose, 30 days post the fourth dose, and 24–30 months post the first dose. **c)** Plasma and PBMC were separated from blood samples and assessed for spike-specific humoral and cellular B responses. Spike-specific antibodies and ACE-2/RDB binding inhibition activity were assessed by ELISA and SARS-CoV-2 sVNT assay at all time points, while RBD-specific B cells were characterized in blood collected after the second, third and fourth vaccine doses, and after 24–30 months post the first dose.

assessing the antibody and B cell responses is reported in Supplementary Figures S1 and S2, respectively.

Among participants who were immunocompromised, the median time since transplantation was 6.4 years for hematopoietic stem cell transplant recipients and 9 years for solid organ transplant recipients. The median timing of initiation of dialysis was 5.5 years. The median time since HIV diagnosis was 11.1 years. All

	Study groups	HM[a]	HD	HCT	SOT	PLWH[d]	IC
N participants		160	58	56	198	113	123
Sex	Female n. (%) Male n. (%)	69 (43%) 91 (57%)	24 (41%) 34 (59%)	23 (41%) 33 (59%)	51 (26%) 147 (74%)	20 (18%) 93 (82%)	82 (67%) 41 (33%)
Mean age at enrollment (median) (IQR)		68 (70) (33–91)	71 (73) (66–81)	52 (53) (46–62)	59 (62) (54–67)	52 (53) (46–58)	44 (46) (22–75)
Vaccine booster n (%)	**3rd dose**	38 (24%)	53 (91%)	56 (100%)	188 (95%)	113 (100%)	123 (100%)
	4th dose	n/a	44 (76%)	53 (95%)	160 (81%)	60 (16%)	18 (15%)
	Original wt		100%	100%	88%	79%	85%
	Omicron-adapted		0%	0%	12%	21%	15%
	5th dose	n/a	24 (41%)	n/a	74 (39%)	5 (4%)	n/a
	Omicron-adapted		100%		100%	100%	
Samples post boosters (n)	Post v3	38[b]	53	50	75	33	75
	Post v4	–	44	43	36	8	12
	Year 2	–	32	–	135	64	54
SARS-CoV-2 infection n (%)		n/a	16 (28%)	12 (22%)	95 (48%)	20 (17%)	60 (49%)
Median years from diagnosis/transplantation (IQR)		n/a	5.5 (3.1–10.3)	6.4 (0.2–18.9)	9 (4.2–15)	11.1 (6.3–27.3)	n/a
Immunosuppressive treatment		36 (22%)	3 (5%)	11 (20%)	198 (100%) (48%[c])	0%	n/a

n/a, not applicable. [a]Hematological malignancies group included participants affected by acute myeloid leukemia (AML, 17), chronic lymphocytic leukemia (CLL, 30), lymphoma (54), multiple myeloma (MM, 21) and primary myelofibrosis (MF, 38). [b]Only participants with primary myelofibrosis received a 3rd vaccine dose. [c]In treatment with mycophenolate mofetil (MMF) at 2 years after vaccination. [d]96% of PLWH had a $CD4^+$ T cells count >350/mmc and undetectable viral load.

Table 1: **Characteristics of the study groups.**

SOT participants were on immunosuppressive therapy throughout the study period, with 48% receiving mycophenolate mofetil (MMF). In contrast, only 20% of HCT and 5% of HD participants were on immunosuppressive treatment.

The immunocompetent comparison group included 123 participants, who received the third booster dose (9–10 months after the first dose), while only 15% received the fourth dose (around 9–10 months after the third dose) and none received the fifth. Among participants vaccinated with the fourth dose, 15% received an Omicron-adapted vaccine. Among participants who were immunocompetent, 49% self-reported SARS-CoV-2 infection during the course of the study (Table 1).

Comparison of the spike-specific antibody response elicited by the 2-dose primary vaccination across different groups

Spike-specific IgG titres varied according to the different groups after the 2-dose primary vaccination (Fig. 2a). Participants with AML, HIV or stem cells transplantation produced an IgG response comparable with participants who were immunocompetent, with a median titre of 10,240 (IQR 5120–10240), 20,480 (IQR 10240–40960), 10,240 (IQR 5120–40960) and 20,480 (IQR 10240–40960), respectively, while all the other participants developed a significant lower response ($P < 0.001$ *versus* the comparison group), especially the CLL and SOT groups with median titre of 640 (IQR 280–2240 and 160–2560, respectively) and participants with MM with median titre of 1280 (IQR 960–5120). Individuals affected by lymphoma developed a response with a median titre of 3840 (IQR 320–20480), but they showed the highest range of the spike-specific antibody response from a minimum titre of 20 to a maximum of 163,820. Participants with primary myelofibrosis had median titre of 5120 (IQR 2560–20480), while those undergoing haemodialysis of 2560 (IQR 1280–10240) (Fig. 2a).

The functionality of the spike-specific antibodies detected in plasma after the 2-dose primary vaccination was tested via their ability to bind to the viral RBD, thus blocking the interaction with ACE-2 molecules, employing a sVNT. A different functionality was observed across the different groups, with AML, HCT and PLWH showing similar binding inhibition as IC, while participants with hematological malignancies, undergoing HD and SOT showed reduced capacity to inhibit binding (Fig. 2b; $P < 0.001$ for CLL, MM, Lymphoma, MF, HD and SOT *versus* IC, according to Fisher test). The percentage of participants with inhibition value ≥ 30% in AML, HCT and PLWH was 78%, 82% and 85%, respectively *versus* 92% in IC, while in CCL, MM, lymphoma, MF, HD and SOT it was only the 26.6%, 14.2%, 50%, 47%, 50.8% and 41.6% of participants, respectively (Fig. 2c; $P < 0.001$ for CLL, MM, Lymphoma, MF, HD and SOT *versus* IC, according to Fisher test).

Impact of booster doses on antibody and spike-specific B cell responses across different groups

Individuals who are immunocompromised have been considered a priority category for booster doses, due to the impaired immune responsiveness to vaccination.

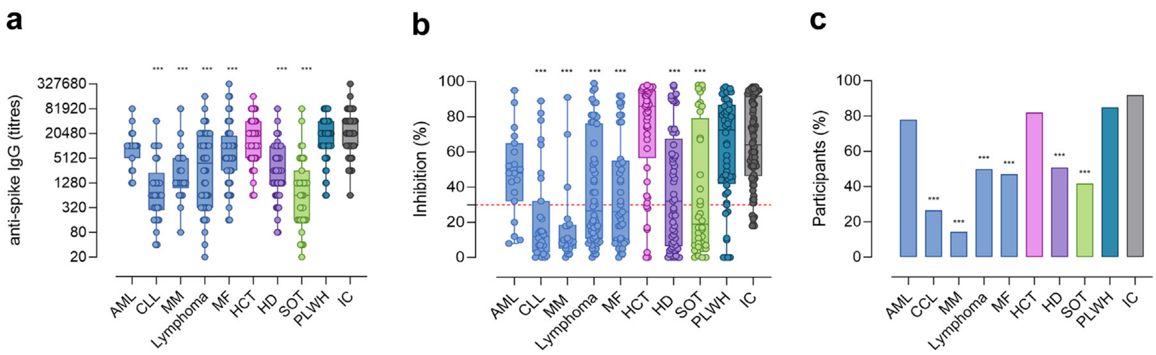

Fig. 2: **Spike-specific antibody response elicited by the 2-dose primary vaccination across different immunocompromised groups.** **a)** Spike-specific IgG titres were assessed in plasma samples collected 10–30 days after the second vaccine dose administration, in the groups of individuals with haematological malignancies (n = 160), hematopoietic stem cell transplantation (HCT, n = 56), undergoing haemodialysis (HD, n = 58), solid organ transplantion (SOT, n = 36), people living with HIV (PLWH, n = 53) and individuals who are immunocompetent (IC, n = 93). The haematological malignancies group included individuals affected by acute myeloid leukaemia (AML, n = 17), chronic lymphocytic leukaemia (CLL, n = 30), multiple myeloma (MM, n = 21), lymphoma (n = 54) and primary myelofibrosis (MF, n = 38). Kruskal–Wallis test, followed by Dunn's post-test for multiple comparisons, was used for assessing statistical differences between immunocompromised groups *versus* IC (***P < 0.001). **b)** sVNT assay performed 10–30 days after the second vaccine dose administration. The red dotted line, placed at 30% inhibition percentage, indicates the threshold of positivity of the assay. Differences in the number of individuals who are immunocompromised and individuals who are immunocompetent positive for the test were assessed using Fisher's exact test (***P < 0.001). **c)** Frequency of individuals with a value of inhibition ≥30% (as described in b).

Here, we longitudinally analysed the effect of the third and fourth booster doses across the different groups in terms of spike specific antibodies and B cell response in comparison with immunocompetent participants.

Booster impact on spike-specific antibody response across different immunocompromised groups

The longitudinal analysis of the spike-specific antibody response upon the third and fourth booster doses was performed and compared across the different groups. For haematological participants the follow up was performed for individuals with myelofibrosis, whose samples were collected until the third vaccine dose.

In all groups the booster doses elicited a strong recall effect, as shown in Fig. 3a. Upon the fourth dose only SOT participants persisted significantly lower compared to IC while in all the other groups the spike-specific IgG titres become similar to the IC group (P < 0.05; P values obtained comparing immunocompromised groups to IC, for each time point, are reported in Supplementary Table S7). To measure the recall effect of the booster dose on the spike-specific antibody levels a "fold change" factor was calculated. The factor was expressed as the ratio between the logarithm of the antibody titres measured after and before each vaccine administration (Fig. 3b). The 2-dose primary vaccination elicited the highest "fold change" factor in the immunocompetent group, which was then progressively lower in PLWH, participants with hematopoietic stem cell transplantation, undergoing haemodialysis, with myelofibrosis, and finally lowest in participants with solid organ transplantation, which showed a very low vaccination effect in terms of antibody rise. The increase due to the booster dose was higher in HD, HCT, PLWH and MF compared to the IC comparison group, demonstrating a higher impact of the booster dose on the immune responsiveness of participants who are immunocompromised *versus* those who are immunocompetent. Only SOT participants remained significantly below the values of all the other groups, showing the lowest fold increase at each booster dose (P < 0.01).

Finally, each booster dose strongly increased the percentage of participants with antibodies capable of binding the viral RBD and blocking its interaction with ACE-2 molecules, reaching levels comparable with IC participants upon the fourth dose (Fig. 3c; P values obtained comparing groups of participants who were immunocompromised to those who were immunocompetent, for each time point, are reported in Supplementary Table S8).

Booster impact on spike-specific B cell response across immunocompromised different groups

Spike-specific memory B cells were analysed by multiparametric flow cytometry, after the 2-dose primary vaccination, the third and fourth doses in all groups of participants who were immunocompromised and in those who were immunocompetent, by multiparametric flow cytometry. Spike-specific memory B cells (hereafter named S^+ MBC), identified among total $CD19^+$ non-*naïve* cells (excluding the $CD27^-IgD^+$) are shown in Fig. 4a, with a representative dot plot for each group. Since the detection and measurement of S^+ MBC is sensitive to multiple factors, we compared each group

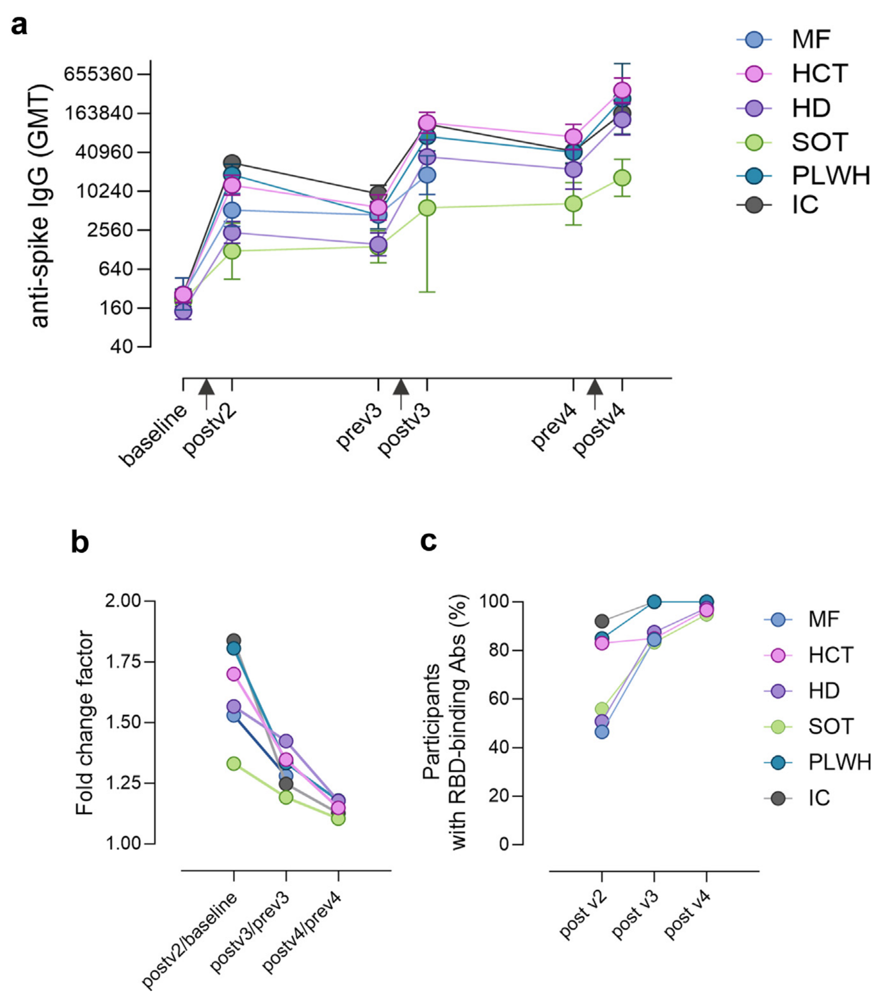

Fig. 3: **Longitudinal analysis of spike-specific antibody response across different groups of individuals who are immunocompromised upon booster doses administration. a)** Longitudinal analysis of spike-specific IgG titres in plasma samples collected before and after each vaccine dose (arrows), in the groups of individuals with primary myelofibrosis (MF), hematopoietic stem cell transplantation (HCT), undergoing haemodialysis (HD), solid organ transplantation (SOT), people living with HIV (PLWH) and individuals who are immunocompetent (IC). The number of samples for each time point in each groups is reported in Supplementary Figure S1. **b)** Fold change Factor calculated as the ratio between the logarithm of the antibody titres measured after and before each vaccine dose administration. **c)** Frequency of individuals with a value of inhibition ≥30% detected post the second, third and fourth doses (excepted MF), for each groups.

with a subset of participants who were immunocompetent specifically selected for matching age, sex and time of blood collection. The frequency of total CD19$^+$ B cells detected in each group is reported in Supplementary Figure S3. Frequencies of S$^+$ MBC (respect to total CD19$^+$ cells) assessed in each group after the second, third and fourth doses are shown in comparison to frequencies in IC in Fig. 4b. The ratio between the median frequency of S$^+$ MBC in each group *versus* the respective comparison group of participants who were immunocompetent is visualized in the heatmap in Fig. 4c. Only PLWH generated a S$^+$ MBC response comparable to participants who were immunocompetent since the 2-dose primary vaccination (0.285 [0.210–0.448] *versus* 0.250 [0.150–0.400]), that was maintained after the third and the fourth dose (Fig. 4 b,c). MF, HCT and HD presented significantly lower rate of S$^+$ MBC compared to the respective comparison group after the first two doses ($P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.001$, respectively, Fig. 4b). These frequencies were extremely lower compared to the IC comparison group, with a ratio 0.32 in MF, 0.51 in HCT and 0.16 in HD (Fig. 4c). Nevertheless, the third booster dose strongly increased the frequency of S$^+$ MBC and values with a ratio >0.72 were measured in all groups, except SOT. Slightly higher ratios were observed after the fourth vaccine dose (Fig. 4c).

To further dissect the B cell response and profile the different subsets of S$^+$ MBC, the frequency of Ig-switched (CD27$^+$ IgD$^-$), unswitched (CD27$^+$ IgD$^+$) and

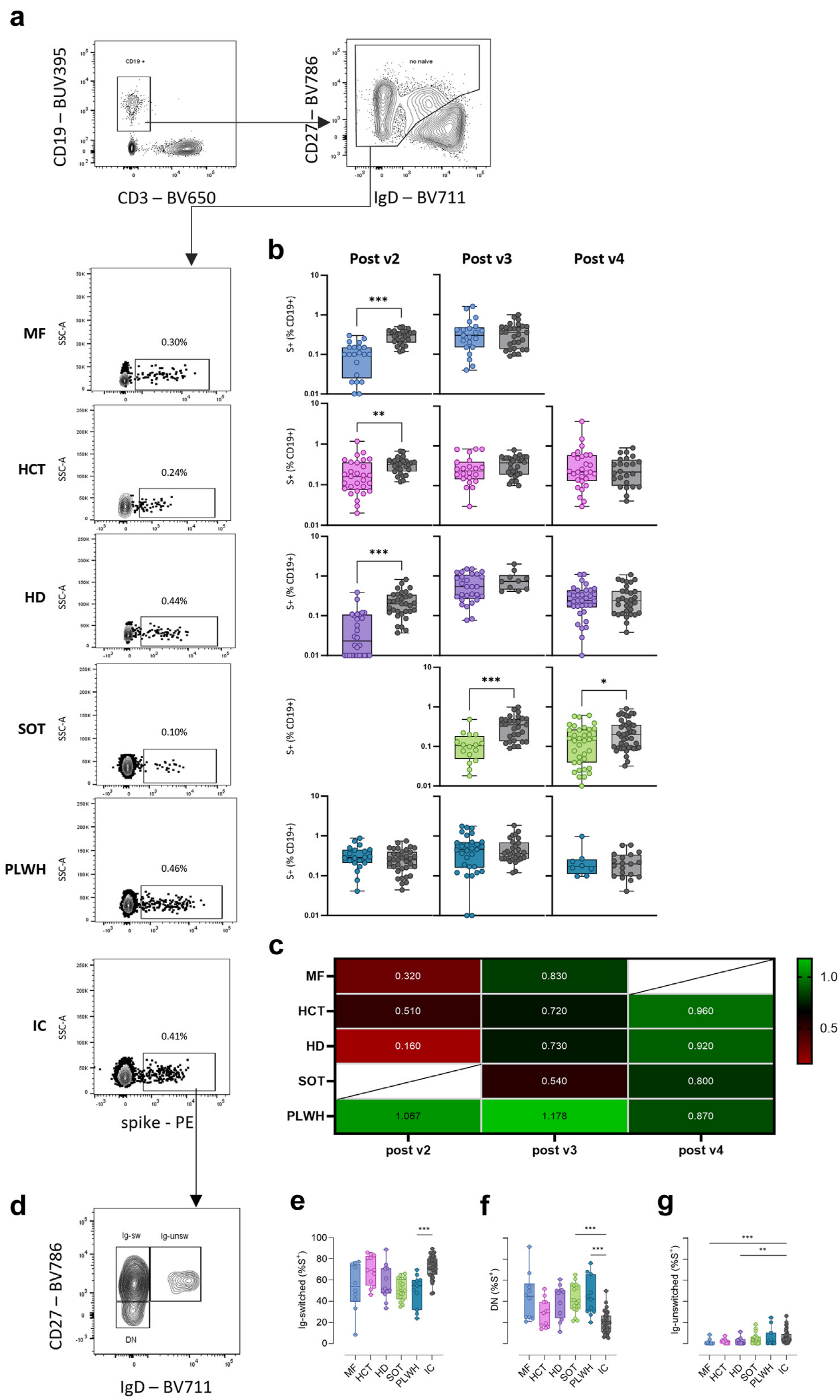

Fig. 4: **Spike-specific B cells across different groups of immunocompromised individuals upon booster doses administration**. **a)** Gating strategy for identifying CD19+ non-*naïve* spike-specific B cells (S+ MBC) by multiparametric flow cytometry, in each group (representative dot

double negative ($CD27^-$ IgD^-) cells (Fig. 4d) was compared across the immunocompromised groups (Fig. 4 e-g). While the Ig-switched phenotype was dominant in all groups, PLWH showed a significantly lower frequency compared to IC (Fig. 4e), while the DN subset was significantly more expressed in both PLWH and SOT participants (Fig. 4f). Ig-unswitched S^+ MBC was the subset less detectable in all groups, especially among haematological participants (MF), HCT, HD and SOT (Fig. 4g).

The frequency of S^+ MBC significantly correlated with S^+ IgG titres assessed upon the subsequent vaccine dose (Supplementary Table S9).

Long-term persistence of the immune response across different immunocompromised groups

To evaluate the long-term persistence of the immune response elicited by the vaccine administration and the impact of breakthrough infection, we stratified participants in infected and non-infected, and we followed up the humoral and memory B cell responses, specific for both wt and Omicron BA.2 RBD proteins, in the two subgroups. The number of participants who were infected was 20 among PLWH, 70 among SOT, 12 among HD and 31 among IC participants, while non-infected participants were 44 among PLWH, 65 among SOT, 32 among HD and 23 among IC participants. All participants who reported infection had mild disease symptoms, that did not require hospitalization or result in significant respiratory distress.[25]

Wt and Omicron BA.2 specific IgG persistence in participants who were infected and non-infected

PLWH, SOT and HD participants showed a persistent wt spike-specific antibody response at 24–30 months from the first dose administration (Fig. 5a). Stratification between participants who were infected and non-infected did not highlight significant differences among spike-specific titres within each group, even though a significantly higher median value was observed among infected IC compared to uninfected IC ($P < 0.05$). PLWH and SOT who reported breakthrough infection had significantly lower IgG titres compared to infected IC participants (Fig. 5a). The IgG response was significantly lower also against BA.2-RBD in infected PLWH and SOT participants compared to infected IC (Fig. 5b).

All groups presented high rates of participants with antibodies capable of binding both the wt and the BA.2 variant and blocking their interaction with ACE-2 (Fig. 5 c,d). In all groups, the percentages of participants with values ≥ 30% threshold were higher than 93% and 82% against the wt and BA.2 RBD, respectively, with no statistical differences detected between infected and non-infected groups. Only infected SOT participants showed significantly reduced inhibition of binding compared to infected IC ($P < 0.01$).

Wt and Omicron BA.2 specific memory B cells in participants who were infected and non-infected

The analysis of RBD-specific memory B cell persistence was performed considering three different sub-populations: B cells recognizing the original wt RBD (wt^+RBD), cells reactive against both wt and Omicron BA.2 RBD ($wt^+/BA.2^+$ RBD), and cells that specifically recognized only the BA.2 RBD variant ($BA.2^+$ RBD; Fig. 6a). PLWH and IC showed a similar distribution among the three RBD^+ subpopulations, with a lower portion of cell $BA.2^+$ RBD and a higher portion of B cells reactive against the original wt antigen (wt^+ RBD and $wt^+/BA.2^+$ RBD, Fig. 6b). HD and SOT showed instead a higher portion of $BA.2^+$ RBD B cells (Fig. 6b). To investigate the possible impact of infection, we split each group into participants who were infected and non-infected, but no significant differences were detected between the respective subgroups (Fig. 6c–e). A significant lower frequency of wt^+ RBD-specific B cells was measured only in infected SOT group (Fig. 6c; $P < 0.05$ *versus* infected IC) while $wt^+/BA.2^+$ RBD-specific B cells were lower in infected SOT participants when compared to non-infected HD, PLWH and infected IC ($P < 0.001$ *versus* HD, $P < 0.01$ *versus* PLWH and $P < 0.05$ *versus* infected IC) and in non-infected SOT participants compared to HD and PLWH ($P < 0.01$ *versus* HD and $P < 0.05$ *versus* PLWH; Fig. 6d). The analysis of the B cells reacting only with the Omicron BA.2 RBD showed a significant higher frequency among infected HD participants compared to participants who were immunocompetent and PLWH (Fig. 6e; $P < 0.05$ infected HD *versus* PLWH, infected PLWH, IC and infected IC). The analysis highlights the crucial role of the Omicron-adapted vaccines in stimulating a higher BA.2-specific B cell response. Indeed, participants who were immunocompetent and most of PLWH, did not receive the

plot). **b)** Frequency of S^+ MBC cell (calculated respect to total $CD19^+$cells) detected in each group of immunocompromised patients and the matched immunocompetent comparison group, after the second, third and fourth vaccine doses. **c)** Ratio between the median value of S^+ MBC frequency (calculated respect to total $CD19^+$ cells) detected in the specific groups and the matched immunocompetent comparison group, after the second, third and fourth vaccine doses reported as heatmap. **d)** Gating strategy for identifying Ig-switched ($CD27^+$ IgD^-), Ig-unswitched ($CD27^+$ IgD^+), and double negative DN ($CD27^-$ IgD^-) subsets among S^+ MBC. **e–g)** Frequency of Ig-switched (e), DN subsets (f) and Ig-unswitched (g) S^+ MBC assessed post the third dose in each groups of immunocompromised patients. Kruskal–Wallis test, followed by Dunn's post-test for multiple comparisons, was used for assessing statistical differences between groups of individuals who were immunocompromised and IC comparison group (*$P < 0.05$; ***$P < 0.001$).

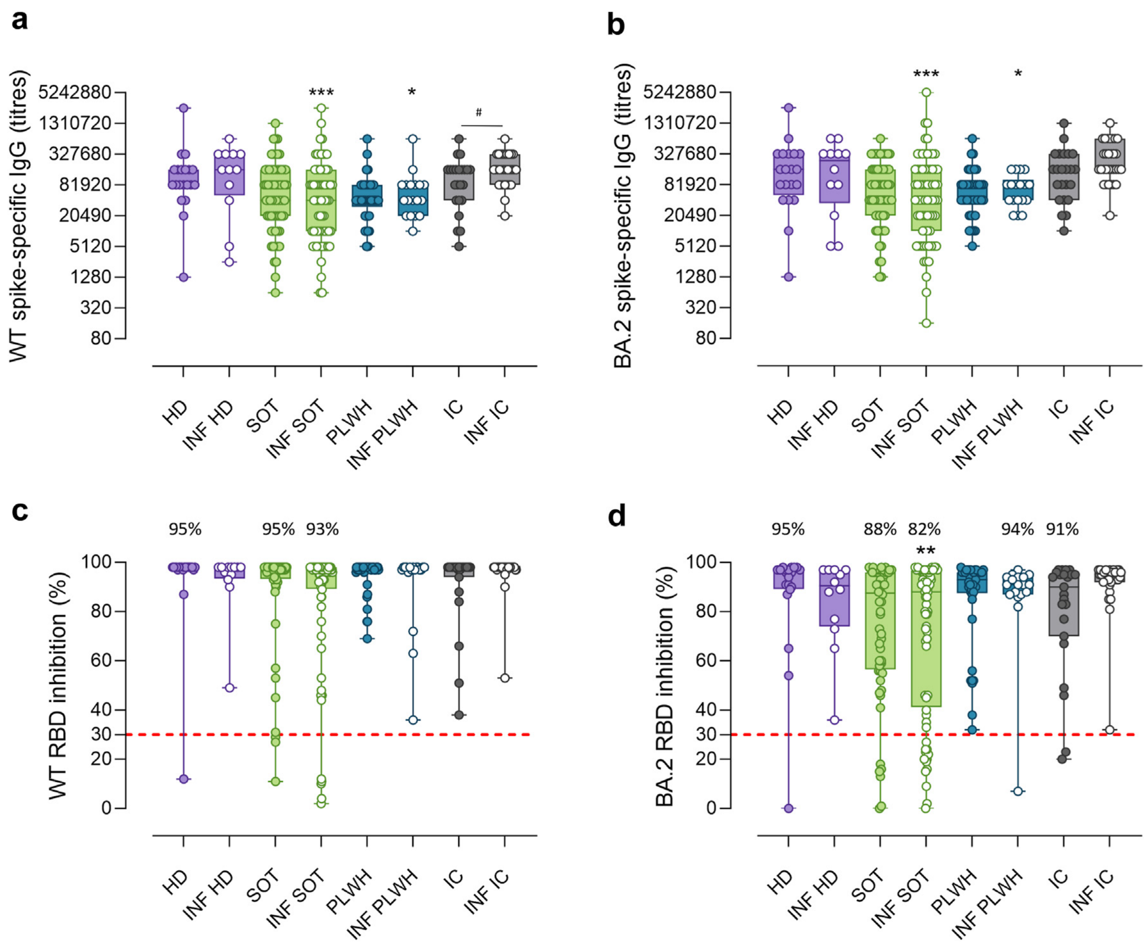

Fig. 5:* Long term analysis of spike-specific antibody response across different groups of immunocompromised individuals. a–b)** IgG titres specific for spike antigen from wild type (A) and BA.2 Omicron variant (B), assessed at 24–30 months, in the groups of individuals undergoing haemodialysis (HD, n = 32), with solid organ transplantation (SOT, n = 135), people living with HIV (PLWH, n = 64) and individuals who are immunocompetent (IC, n = 54), divided into SARS-CoV-2 non-infected (close circles; HD, SOT, PLWH and IC) and infected (open circles; INF HD, INF SOT, INF PLWH and INF IC) individuals. Analysis was performed in blood samples collected from immunocompromised individuals vaccinated with fourth or five doses, and in immunocompetent individuals vaccinated with three or fourth doses. Kruskal–Wallis test, followed by Dunn's post-test for multiple comparisons, was used for assessing statistical differences between groups of infected immunocompromised and infected controls or non-infected immunocompromised individuals and non-infected controls (*P < 0.05; ***P < 0.001); Mann–Whitney was used for assessing statistical differences between infected and non-infected individuals within each groups ($^{\#}$ <0.05). **c–d)** sVNT assay specific for spike antigen from wild type (C) and BA.2 Omicron variant (D) performed at 24–30 months. The red dotted line, placed at 30% inhibition percentage, indicates the threshold of positivity of the assay. Frequency of individuals with a positive inhibition value is reported above. Differences in the number of individuals positive for inhibition test were assessed between immunocompromised groups and IC within infected and non-infected groups and between infected and non-infected samples within each groups using Fisher's exact test (P < 0.001).

new Omicron-adapted vaccine formulations, while most of the HD and SOT participants received Omicron-adapted vaccines for the fourth and fifth doses (Supplementary Tables S3–S6).

Phenotypes of RBD-specific B cells were compared in participants who were infected and non-infected among the different groups by combining manual gating and t-SNE dimensionality reduction. t-SNE was used to display in a bi-dimensional space different cell subsets according to the expression of CD27, CD21, IgD, IgM, IgA and IgG (Fig. 7a). According to the markers expression, activated memory ($CD27^{+}CD21^{-}$), resting memory ($CD27^{+}CD21^{+}$), atypical/DN2 ($CD27^{-}CD21^{-}$) and DN1 ($CD27^{-}CD21^{+}$) B cell subsets, and their isotype switching in IgG^{+}, IgA^{+}, IgM^{+} or $IgD^{+}IgM^{+}$ cells, were distributed in different regions of the t-SNE map (Fig. 7b).

The distribution of antigen-specific B cell subsets was then visualized for the three subpopulations (wt^{+} RBD, both $wt^{+}/BA.2^{+}$ RBD and $BA.2^{+}$ RBD B cells) in each group, split into infected and non-infected

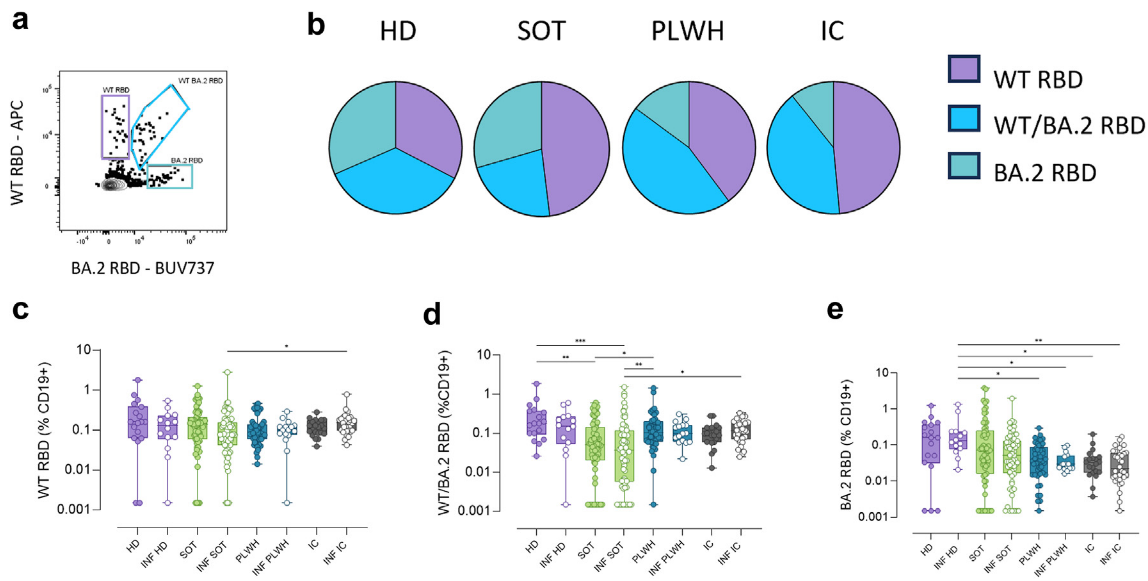

Fig. 6: **Wt and Omicron BA.2 specific memory B cells across different groups of immunocompromised individuals. a)** Gating strategy for identifying wt$^+$ RBD, wt$^+$/BA.2$^+$ RBD, and BA.2$^+$ RBD. **b)** Pie chart showing the distribution of wt$^+$RBD, wt$^+$/BA.2$^+$ RBD, and BA.2$^+$ RBD MBC among groups of individuals undergoing haemodialysis (HD, n = 32), solid organ transplanted (SOT, n = 125), people living with HIV (PLWH, n = 62) and immunocompetent comparison group (IC, n = 54). **c–e)** Frequency of wt$^+$ RBD (c), wt$^+$/BA.2$^+$ RBD (d) and BA.2$^+$ RBD (e) MBC in the groups of individuals undergoing haemodialysis (HD), with solid organ transplantation (SOT), people living with HIV (PLWH) and individuals who are immunocompetent (IC), separated between non-infected (close circles, HD, SOT, PLWH and IC) and infected (open circles, INF HD, INF SOT, INF PLWH and INF IC) individuals. Kruskal–Wallis test, followed by Dunn's post-test for multiple comparison, was used to assess statistical differences among not infected individuals under different groups, and among infected individuals under different groups (*P < 0.05; **P < 0.01; ***P < 0.001).

subgroups (Fig. 7c). Different B cell subsets were observed among B cell clones recognizing the different antigens. Most of the B cells reactive for the wt RBD (wt$^+$RBD and wt$^+$/BA.2$^+$ RBD) were IgG$^+$ resting MBC, while the BA.2$^+$ RBD B cells had a higher proportion of IgG$^+$ activated and atypical/DN2, and IgA$^+$ skewed MBC (Fig. 7c). B cells recognizing only the BA.2 RBD showed a lower portion of IgG$^+$ resting MBC.

These differences were statistically significant by applying a non-parametric Dunn's test (Supplementary Table S10). To better visualize the significant differences between groups, the frequencies of B cell subsets were displayed as radar plots (Fig. 7d). The cell subsets corresponding to IgG$^+$ (split in activated, resting and DN), IgA$^+$ and IgM$^+$ are reported in the radar axis, with the respective minimum and maximum median values for each population (Fig. 7d, left panel). Since no significant differences were detected between participants who were infected and non-infected for most phenotypes observed, the two subgroups were merged within each group. BA2$^+$ RBD-specific B cells in SOT group showed a high number of IgG$^+$ activated MBC and DN2 subsets, while the HD group showed a higher frequency of IgA$^+$ B cells (Fig. 7d, right panel). Among IC participants, a subset of IgM$^+$ BA.2-specific B cells was observed, while B cells reactive against wt RBD (wt$^+$RBD and wt$^+$/BA.2$^+$RBD) showed a predominance towards IgG$^+$ resting MBC (Fig. 7d). All together the phenotypical analysis of B cells specific for wt and BA.2 RBD, further demonstrates that participants vaccinated with the Omicron-adapted vaccine formulations better develop a B cell response specific for the new variant, and the vaccine impact seems to be stronger than the breakthrough infection.

Discussion

In this longitudinal single-center prospective study, we analysed the immune responsiveness to SARS-CoV-2 vaccination across 5 different groups of individuals who were immunocompromised in comparison to a group of participants who were immunocompetent. Participants were enrolled at a single clinical site, following identical experimental protocols, and subjected to immunological assays conducted in a unified laboratory setting. This approach has been fundamental to yield comparable data useful for addressing knowledge gaps regarding the immune response to SARS-CoV-2 vaccination in diverse immunocompromised patient populations. Open questions regarding the immunogenicity of the 2-dose primary vaccination, impact of the booster doses, role of the new Omicron-adapted vaccine formulations and the long-term persistence not only of spike-specific antibodies but also of spike-specific memory B cells were

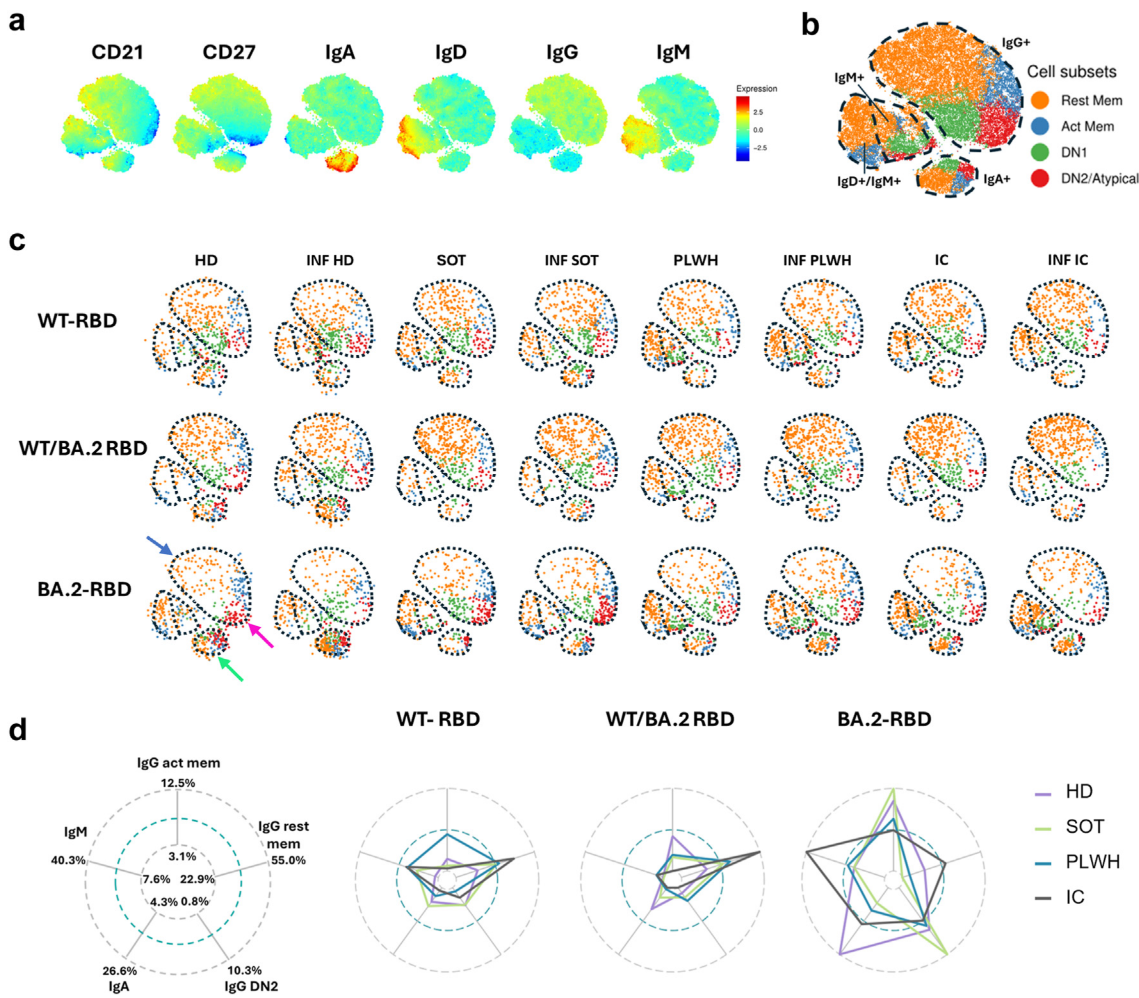

Fig. 7: **t-SNE visualization of RBD-specific B cell phenotypes across different groups of immunocompromised individuals**. wt$^+$ RBD, wt$^+$/BA.2$^+$ RBD, and BA.2$^+$ RBD B cells were analysed using a t-SNE dimensionality reduction approach. **a)** Expression of analysed markers (z-score) is visualized as a color scale from low (blue) to high (red). **b)** B cell populations gated as activated memory (CD27$^+$CD21$^-$), resting memory (CD27$^+$CD21$^+$), atypical/DN2 (CD27$^-$CD21$^-$), and DN1 (CD27$^-$CD21$^+$) are visualized in different t-SNE map regions related to different Ig expressions. **c)** Different distribution of B cell phenotypes across the groups of individuals undergoing haemodialysis (HD), with solid organ transplantation (SOT), people living with HIV (PLWH) and individuals who are immunocompetent (IC), separated between non-infected (HD, SOT, PLWH and IC) and infected (INF HD, INF SOT, INF PLWH and INF IC) individuals. Regions (dashed lines) and colours are defined in panel B. IgG$^+$ resting MBC (blue arrow), IgG$^+$ activated and atypical/DN2 (pink arrow) and IgA$^+$ MBC (green arrow) are shown in the representative HD t-SNE map. **d)** Selected subsets (significantly different between groups or antigens as reported in Supplementary Table S6) were visualized in radar plots. Left panel reports the minimum and maximum values for each axis. The analysis has been performed for wt$^+$ RBD, wt$^+$/BA.2$^+$ RBD, and BA.2$^+$ RBD B cells pooling together infected and non-infected individuals for each groups.

addressed in this study by comparing different pathologies with each other and against the immunocompetent comparison group in a real-world setting. Considering the circulation of the virus and its variants in these years, we also took in consideration the impact of the SARS-CoV-2 infection on the immune response detected at 2 years after vaccination.

This study highlights some critical aspects in the vaccination of individuals who are immunocompromised, such as i) the heterogeneous immune responsiveness among individuals affected by different pathologies, ii) the critical role of boosting the immune system of individuals who are immunocompromised to produce an immune response similar to the one elicited with the 2-dose primary vaccination in individuals who are immunocompetent, iii) the critical role of Omicron-adapted vaccine formulations in stimulating the BA.2 RBD-specific B cell response, and iv) the long-term persistence of the immune response in individuals who are immunocompromised, although some groups–

such as HD and SOT recipients–include individuals who remain low responders despite receiving repeated booster doses.

Heterogeneity in the humoral responses among the different groups was observed mainly after the 2-dose primary vaccination. Participants with haematological malignancies, especially CLL and MM, solid organ transplantation and undergoing haemodialysis were the lower responders, while those with stem-cell transplantation and PLWH developed IgG titres similar to participants who were immunocompetent already after two vaccines doses.

In PLWH, this can be due to the effective ART treatment, indeed less than 5% of them had CD4+ T cell count <200/mmc, around 12% of participants had CD4+ T cell count in the 350–500/mmc range and 83% had CD4+/mm > 500. Moreover, 43% of them presented an optimal immunological recovery (OIR).[26] In participants who underwent hematopoietic stem-cell transplantation the seroconversion, similar to that seen in the IC group, could be attributed to the time elapsed between allogeneic hematopoietic cell transplantation and the administration of the first SARS-CoV-2 vaccine dose. Indeed, in our group, this time was an average of 6 years (range: 0.2–18.6 years), which is longer compared to other studies.[27] Moreover, a relatively low frequency (20%) of participants was on immunosuppressive therapy.

Participants undergoing haemodialysis were extremely heterogenous in the response to the 2-dose primary vaccination, with only about half of them developing a spike-specific antibody response after the first two vaccine doses.

The most heterogenous group encompassed participants with haematological malignancies affected by different disorders, such as acute myeloid leukaemia, chronic lymphocytic leukaemia, lymphoma, multiple myeloma and myeloproliferative neoplasms. These pathologies, and the stage of disease, differently impacted on the immune response observed after the 2-dose primary vaccination.[28] Indeed, while the immune response of participants affected by acute myeloid leukaemia was not significantly different compared to those who were immunocompetent (except for 4 participants with active disease), individuals with chronic lymphocytic leukaemia and participants with B-cell neoplasms showed a strongly lower humoral response. As already reported, the therapeutic treatment, especially with drugs like anti-CD20 mAb (i.e Rituximab), profoundly affects the clinical, serological and long term immune response to infection and vaccination,[29–31] and it is critical for antibody seroconversion.[32] Concerning the myeloproliferative disorders, the wide use of ruxolitinib treatment[33] seems to impact on vaccine immunity after the 2-dose primary vaccination,[5,34,35] while the booster dose reduces the differences compared to untreated individuals.[4,36]

Administration of the third dose significantly increased the percentage of responder participants in all groups, except in SOT. Antibodies reached values that were similar to the ones elicited by the 2-dose primary vaccination in individuals who were immunocompetent.

In this study we demonstrated and monitored the induction not only of spike-specific antibodies, but also of spike-specific memory B cells. All the groups of participants who were immunocompromised, except PLWH under ART, produced a lower amount of circulating antigen-specific B cells compared to immunocompetent participants after the 2-dose primary vaccination. Nevertheless, the third and subsequent booster doses efficiently expanded the pool of the antigen-specific immune B cells in all groups, except for SOT, eliciting memory B cells similar to the values detected in participants who were immunocompetent. The spike-specific B cells analysed after the third dose showed a similar phenotypical profile across the different groups, with a predominance of Ig-switched MBC.[12,37] PLWH was the only one group that developed a different phenotypical distribution upon vaccination, with a higher frequency of DN $CD27^{-}$ IgD^{-} spike-specific B cells, as observed in our previous study.[6] This can be the consequence of known B cells perturbances,[38] that also impair the generation of long-term memory B cells and the germinal centre reaction,[39] Nevertheless, in a longitudinal study currently performed in this group, we observed a reduction of DN cells concomitant with an increase of Ig-switched subset upon the fourth and the fifth doses (Polvere et al., in preparation).

The analysis of RBD-specific B cells performed at longer time points highlighted the crucial impact of the new Omicron-adapted vaccines in priming a BA.2 RBD-specific immune response. Indeed, participants undergoing haemodialysis and solid organ transplantation, who prevalently received the Omicron BA.4/BA.5 or XBB.1.5-adapted vaccines, developed a higher BA.2 RBD-specific B cell response compared to participants who were immunocompetent, and mostly vaccinated with original strain-vaccine. The phenotypic analysis of the BA.2 RBD-specific clones showed a prevalence of IgG^{+} activated ($CD27^{+}CD21^{-}$) MBC, and atypical/DN2 ($CD27^{-}CD21^{-}$). This phenotypic profile resembled the one specific for wt RBD monitored in immunocompetent participants after the 2-dose primary vaccination.[12] In immunocompetent participants and PLWH, also after Omicron breakthrough infections, we observed a predominance of wt RBD and wt^{+}/$BA.2^{+}$ RBD reactive memory B cells, rather than a priming of Omicron-specific *naive* B cells. This can be due to persisting immune imprinting elicited by repeated original spike exposures, that might compromise elicitation of immunity against new SARS-CoV-2 variants.[40] Indeed, while most of the B cell response shifted towards wt^{+} RBD IgG^{+} resting MBC, the $BA.2^{+}$ RBD-specific B cell response showed a predominance of cells still IgM^{+}.

Yet, having information on the memory B cell response in individuals who are immunocompromised after vaccination with the novel mRNA-based vaccine platform is extremely important, as most of the studies performed in individuals who are immunocompromised mainly assessed only the humoral response, especially with the Omicron-adapted vaccines.[9,10]

Concerning the persistence of the antibody immune response, mRNA vaccination elicited a long-term response in participants who were immunocompromised, still measurable 2 years after the first vaccine administration. The comparison of the long-term IgG response between participants who have been infected and non-infected conducted within each group did not show significant differences, although a slightly higher IgG response was observed among the IC group. Since, in most cases of infection, individuals did not receive subsequent booster doses—considering the infection itself as a source of antigen exposure—this could explain the similar immune responses observed between the infected and non-infected subgroups. To note, in SARS-CoV-2-infected and vaccinated SOT and PLWH subgroups the spike-specific IgG levels measured 2 years after vaccination were significantly lower compared to infected and vaccinated IC. This suggests that even though PLWH had successfully responded to vaccination since the first dose, the long-term persistence of IgG is impaired compared to IC. Despite other works have described comparable decline in humoral response in PLWH and individuals who are immunocompetent at shorter time points after mRNA SARS-CoV-2 vaccination,[41,42] reduced immune persistence has been registered for several other vaccines.[43] This may be due to $CD4^+$ T cells alteration related to HIV infection, with consequent impairment of follicular T helper cells, necessary for the generation of both long-term memory B cells and long-lived plasma cells.[44,45]

In HD and SOT groups the long-term persistence of low responsiveness was observed. This can be due to multiple factors, the most important of which are the immunosuppressive treatment with MMF, older age, the time elapsed since transplant and comorbidities.[46–48] MMF anti-rejection activity directly involves B cells, inhibiting cell activation and plasma cells differentiation.[49]

These specific cases should be treated with alternative approaches, such as monoclonal antibodies, instead of further vaccine booster doses, during a pandemic. Interestingly, despite some low responders experienced breakthrough SARS-CoV-2 infections, none of them suffered severe COVID-19 and symptoms were always mild. It is important to note that most infections occurred in the Omicron era, when the circulating virus was considered less aggressive compared to previous variants.[50] However, although not addressed in this work, we can speculate that antigen-specific T cells may have guaranteed some protection in people with lower antibody titres or affected by B cell immunodeficiencies, as also observed in other works.[15,51]

The study has some limitations that should be considered. As a single-center clinical investigation, the findings may not be fully generalizable, and local factors could influence the results. Additionally, variations in sample sizes across groups and time points may impact the statistical power of the analyses. Both the hematological malignancy and hematopoietic cell transplant groups did not continue through the long-term follow-up, and the hematological group had significant discontinuity in blood sampling and withdrew after the third vaccine dose. Furthermore, demographic and clinical variables such as the age, gender and administration of immunosuppressive treatment were not adjusted for the antibody titre measurements due to the limited sizes of the subgroups. Additionally, the study focuses exclusively on individuals vaccinated with mRNA vaccines, in accordance with the national guidelines, and do not account for the impact of other vaccine formulations. Finally, due to limitations in blood sample availability, the study focussed on spike-specific B cell responses, without analysing the T cell response which has shown variable functional quality across different individuals who are immunocompromised[15] and warrants further in-depth investigation.

In conclusion, developing specific vaccination strategies for different medical conditions ensures that vulnerable populations receive optimal protection against vaccine-preventable diseases, thus contributing to overall public health and well-being. The critical aspects highlighted by our study—heterogenous response, impact of booster doses, role of Omicron adapted-vaccines, and persistence of low/non-responders–should be carefully considered for vaccination schedules specifically tailored for each pathology and not generalized for individuals who are immunocompromised. Clinical conditions of individuals, such as stage of disease, immunosuppressive treatment and combination of multiple therapies, have shown to strongly impact the vaccine immune responsiveness, and should always be considered by clinicians. While for PLWH on ART with restored $CD4^+$ T cell count and undetectable viral load, as well as for HCT with a restored and functioning immune system, a single booster dose may be sufficient to elicit an immune response similar to individuals who are immunocompetent, for HD and SOT recipients repeated booster administration appears to be necessary. Nevertheless, for individuals who are low/non-responders alternative approaches to vaccination, such as monoclonal antibodies adapted to circulating variants or specific for highly conserved regions,[52,53] should be considered. Updated variant-adapted booster vaccines should be recommended for all individuals who are immunocompromised, in order to promote a B cell response specific for the circulating viral variants.

Contributors

AC, FM, MF, GP and DM conceptualized the study; AS, MT, AL, FP, MB, AB, GG, DB, SB, MF, FM provided clinical data and enrolled participants; EP, FF, JP, SL, CC, SC, GP processed the samples and carried out the immunological analysis. AC, EP, FF, JP, and SL analysed the data. AC, DM and FM supervised the study. EP, FF and JP accessed and verified the data. AC wrote the original draft. All the authors read and approved the final version of the manuscript. DM provided financial support.

Data sharing statement

Data available upon request.

Ethical statement

The study was performed in compliance with all relevant ethical regulations and the protocol were approved by local Ethical Committee for Clinical experimentation of Regione Toscana Area Vasta Sud Est (CEAVSE), protocol code 19,479 for PATOVAC v1.0 of 03 Mar 2021, approved on 15 Mar 2021 and protocol code 18,869 IMMUNO_COV v1.0 of 18 Nov 2020, approved on 21 Dec 2020.

Informed consent statement

Informed consent was obtained from all participants involved in the study.

Declaration of interests

MB has received honoraria and support for attending meetings from NOVARTIS, ABVVIE, INCYTE.

Acknowledgements

We would like to thank the Nursing staff of the clinics for collecting blood. We thank all the volunteers and the individuals who participated to the study.

Funding: This work was supported by funds from the Department of Medical Biotechnologies of the University of Siena, and from EU within the NextGenerationEU-MUR PNRR Tuscany Health Ecosystem (Project no ECS00000017-THE).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.105577.

References

1 Farooq PD, Sherman KE. Hepatitis B vaccination and waning hepatitis B immunity in persons living with HIV. *Curr HIV AIDS Rep*. 2019;16:395–403.
2 Sam R, Rankin L, Ulasi I, et al. Vaccination for patients receiving dialysis. *Kidney Med*. 2024;6:100775.
3 Pettini E, Ciabattini A, Pastore G, et al. A third dose of mRNA-1273 vaccine improves SARS-CoV-2 immunity in HCT recipients with low antibody response after 2 doses. *Blood Adv*. 2022: 2021006599.
4 Fiorino F, Ciabattini A, Sicuranza A, et al. The third dose of mRNA SARS-CoV-2 vaccines enhances the spike-specific antibody and memory B cell response in myelofibrosis patients. *Front Immunol*. 2022;13. https://www.frontiersin.org/articles/10.3389/fimmu.2022.1017863. Accessed September 30, 2022.
5 Fiorino F, Sicuranza A, Ciabattini A, et al. The slower antibody response in myelofibrosis patients after two doses of mRNA SARS-CoV-2 vaccine calls for a third dose. *Biomedicine*. 2021;9:1480.
6 Polvere J, Fabbiani M, Pastore G, et al. B cell response after SARS-CoV-2 mRNA vaccination in people living with HIV. *Commun Med (Lond)*. 2023;3:13.
7 Teh JSK, Coussement J, Neoh ZCF, et al. Immunogenicity of COVID-19 vaccines in patients with hematologic malignancies: a systematic review and meta-analysis. *Blood Adv*. 2022;6:2014–2034.
8 Garner-Spitzer E, Wagner A, Gudipati V, et al. Lower magnitude and faster waning of antibody responses to SARS-CoV-2 vaccination in anti-TNF-α-treated IBD patients are linked to lack of activation and expansion of cTfh1 cells and impaired B memory cell formation. *EBioMedicine*. 2023;96:104788.
9 Solera JT, Ierullo M, Arbol BG, et al. Bivalent COVID-19 mRNA vaccine against omicron subvariants in immunocompromised patients. *Lancet Infect Dis*. 2023;23:e266–e267.
10 Cossmann A, Hoffmann M, Stankov MV, et al. Immune responses following BNT162b2 XBB.1.5 vaccination in patients on haemodialysis in Germany. *Lancet Infect Dis*. 2024;24:e145–e146.
11 Ciabattini A, Pastore G, Fiorino F, et al. Evidence of SARS-Cov-2-specific memory B cells six months after vaccination with BNT162b2 mRNA vaccine. *Front Immunol*. 2021;12:740708.
12 Ciabattini A, Pastore G, Lucchesi S, et al. Trajectory of spike-specific B cells elicited by two doses of BNT162b2 mRNA vaccine. *Cells*. 2023;12:1706.
13 Mudd PA, Minervina AA, Pogorelyy MV, et al. SARS-CoV-2 mRNA vaccination elicits a robust and persistent T follicular helper cell response in humans. *Cell*. 2022;185:603–613.e15.
14 Boyd MAA, Carey Hoppe A, Kelleher AD, Munier CML. T follicular helper cell responses to SARS-CoV-2 vaccination among healthy and immunocompromised adults. *Immunol Cell Biol*. 2023;101:504–513.
15 Gao Y, Cai C, Wullimann D, et al. Immunodeficiency syndromes differentially impact the functional profile of SARS-CoV-2-specific T cells elicited by mRNA vaccination. *Immunity*. 2022;55:1732–1746.e5.
16 Pettini E, Ciabattini A, Fiorino F, et al. Spike-specific memory B cell response in hematopoietic cell transplantation recipients following multiple mRNA-1273 vaccinations: a longitudinal observational study. *Vaccines*. 2024;12:368.
17 Chen P, Bergman P, Blennow O, et al. Real-world assessment of immunogenicity in immunocompromised individuals following SARS-CoV-2 mRNA vaccination: a one-year follow-up of the prospective clinical trial COVAXID. *eBioMedicine*. 2023;94:104700.
18 Barnes E, Goodyear CS, Willicombe M, et al. SARS-CoV-2-specific immune responses and clinical outcomes after COVID-19 vaccination in patients with immune-suppressive disease. *Nat Med*. 2023;29:1760–1774.
19 Wagner A, Garner-Spitzer E, Schötta A-M, et al. SARS-CoV-2-mRNA booster vaccination reverses non-responsiveness and early antibody waning in immunocompromised patients—a phase four study comparing immune responses in patients with solid cancers, multiple myeloma and inflammatory bowel disease. *Front Immunol*. 2022;13. https://doi.org/10.3389/fimmu.2022.889138.
20 Stefanelli P, Trentini F, Petrone D, et al. Tracking the progressive spread of the SARS-CoV-2 omicron variant in Italy, december 2021 to january 2022. *Eurosurveillance*. 2022;27:2200125.
21 Tan CW, Chia WN, Qin X, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. *Nat Biotechnol*. 2020;38:1073–1078.
22 Parks DR, Roederer M, Moore WA. A new "Logicle" display method avoids deceptive effects of logarithmic scaling for low signals and compensated data. *Cytometry Part A*. 2006;69A:541–551.
23 van der Maaten L, Hinton G. Viualizing data using t-SNE. *J Mach Learn Res*. 2008;9:2579–2605.
24 Noether GE. Sample size determination for some common nonparametric tests. *J Am Stat Assoc*. 1987;82:645–647.
25 COVID-19 Treatment Guidelines Panel. Coronavirus disease 2019 (COVID-19) treatment guidelines. National Institutes of Health. Available at: https://www.covid19treatmentguidelines.nih.gov/. Accessed October 3, 2024.
26 Fabbiani M, Borghetti A, Squillace N, et al. Integrase inhibitors use and cytomegalovirus infection predict immune recovery in people living with HIV starting first-line therapy. *JAIDS*. 2021;86:119.
27 Ni B, Yanis A, Dee K, et al. SARS-CoV-2 vaccine safety and immunogenicity in patients with hematologic malignancies, transplantation, and cellular therapies. *Blood Rev*. 2022;56:100984.
28 Sertić Z, Lucijanić M, Bašić-Kinda S, et al. Non-myelofibrosis chronic myeloproliferative neoplasm patients show better seroconversion rates after SARS-CoV-2 vaccination compared to other hematologic diseases: a multicentric prospective study of KroHem. *Biomedicines*. 2022;10:2892.
29 Furlan A, Forner G, Cipriani L, et al. COVID-19 in B Cell-Depleted patients after Rituximab: a diagnostic and therapeutic challenge. *Front Immunol*. 2021;12. https://doi.org/10.3389/fimmu.2021.763412.
30 Pascale SP, Nuccorini R, Pierri T, et al. Evaluation of serological response to anti-SARS-CoV-2 mRNA vaccination in hematological

patients. *Front Immunol*. 2022;13. https://doi.org/10.3389/fimmu.2022.892331.

31 Wagner A, Garner-Spitzer E, Auer C, et al. Breakthrough infections in SARS-CoV-2-vaccinated multiple myeloma patients improve cross-protection against omicron variants. *Vaccines (Basel)*. 2024;12:518.

32 Fattizzo B, Bortolotti M, Rampi N, et al. Seroconversion to mRNA SARS-CoV-2 vaccines in hematologic patients. *Front Immunol*. 2022;13:852158.

33 Elli EM, Baratè C, Mendicino F, Palandri F, Palumbo GA. Mechanisms underlying the anti-inflammatory and immunosuppressive activity of ruxolitinib. *Front Oncol*. 2019;9:1186.

34 Guglielmelli P, Mazzoni A, Maggi L, et al. Impaired response to first SARS-CoV-2 dose vaccination in myeloproliferative neoplasm patients receiving ruxolitinib. *Am J Hematol*. 2021. https://doi.org/10.1002/ajh.26305.

35 Ikeda D, Terao T, Miura D, et al. Impaired antibody response following the second dose of the BNT162b2 vaccine in patients with myeloproliferative neoplasms receiving ruxolitinib. *Front Med*. 2022;9. https://doi.org/10.3389/fmed.2022.826537.

36 Palumbo GA, Cambria D, La Spina E, et al. Ruxolitinib treatment in myelofibrosis and polycythemia vera causes suboptimal humoral immune response following standard and booster vaccination with BNT162b2 mRNA COVID-19 vaccine. *Front Oncol*. 2023;13. https://doi.org/10.3389/fonc.2023.1117815.

37 Pastore G, Polvere J, Fiorino F, et al. Homologous or heterologous administration of mRNA or adenovirus-vectored vaccines show comparable immunogenicity and effectiveness against the SARS-CoV-2 Omicron variant. *Expert Rev Vaccines*. 2024;23:432–444.

38 Hu Z, Luo Z, Wan Z, et al. HIV-associated memory B cell perturbations. *Vaccine*. 2015;33:2524–2529.

39 Ripperger TJ, Bhattacharya D. Transcriptional and metabolic control of memory B cells and plasma cells. *Annu Rev Immunol*. 2021;39:345–368.

40 Tortorici MA, Addetia A, Seo AJ, et al. Persistent immune imprinting occurs after vaccination with the COVID-19 XBB.1.5 mRNA booster in humans. *Immunity*. 2024;57:904–911.e4.

41 Woldemeskel BA, Karaba AH, Garliss CC, et al. Decay of coronavirus disease 2019 mRNA vaccine-induced immunity in people with HIV. *AIDS*. 2022;36:1315–1317.

42 Vergori A, Cozzi-Lepri A, Matusali G, et al. Long term assessment of anti-SARS-CoV-2 immunogenicity after mRNA vaccine in persons living with HIV. *Vaccines*. 2023;11:1739.

43 Kernéis S, Launay O, Turbelin C, Batteux F, Hanslik T, Boëlle P-Y. Long-term immune responses to vaccination in HIV-infected patients: a systematic review and meta-analysis. *Clin Infect Dis*. 2014;58:1130–1139.

44 Chakhtoura M, Fang M, Cubas R, et al. Germinal Center T follicular helper (GC-Tfh) cell impairment in chronic HIV infection involves c-Maf signaling. *PLoS Pathog*. 2021;17:e1009732.

45 Crotty S. Follicular helper CD4 T cells (TFH). *Annu Rev Immunol*. 2011;29:621–663.

46 Subramanian V. Susceptibility to SARS-CoV-2 infection and immune responses to COVID-19 vaccination among recipients of solid organ transplants. *J Infect Dis*. 2023;228:S34–S45.

47 Zong K, Peng D, Yang H, et al. Risk factors for weak antibody response of SARS-CoV-2 vaccine in adult solid organ transplant recipients: a systemic review and meta-analysis. *Front Immunol*. 2022;13:888385.

48 van Gemert J, Steenberg F, van Leer-Buter C, et al. Increasing antibody responses to five doses of SARS-CoV-2 mRNA vaccine in lung transplant patients. *J Clin Med*. 2023;12:4125.

49 Karnell JL, Karnell FG III, Stephens GL, et al. Mycophenolic acid differentially impacts B cell function depending on the stage of differentiation. *J Immunol*. 2011;187:3603–3612.

50 Hyams C, Challen R, Marlow R, et al. Severity of Omicron (B.1.1.529) and Delta (B.1.617.2) SARS-CoV-2 infection among hospitalised adults: a prospective cohort study in Bristol, United Kingdom. *Lancet Reg Health Eur*. 2023;25. https://doi.org/10.1016/j.lanepe.2022.100556.

51 Moss P. The T cell immune response against SARS-CoV-2. *Nat Immunol*. 2022;23:186–193.

52 Stadler E, Chai KL, Schlub TE, et al. Determinants of passive antibody efficacy in SARS-CoV-2 infection: a systematic review and meta-analysis. *Lancet Microbe*. 2023;4:e883–e892.

53 Wang Y, Yan A, Song D, et al. Identification of a highly conserved neutralizing epitope within the RBD region of diverse SARS-CoV-2 variants. *Nat Commun*. 2024;15:842.