

Immunogenicity of a Three-Dose Primary Series of mRNA COVID-19 Vaccines in Patients With Lymphoid Malignancies

Amy C. Sherman,^{1,2,3,4,a} Jennifer L. Crombie,^{2,4,a} ChiAn Cheng,^{4,5,6,7} Michaël Desjardins,^{1,8} Guohai Zhou,¹ Omolola Ometoruwa,¹ Rebecca Rooks,¹ Yasmeen Senussi,^{4,5,6} Mikaela McDonough,² Liliana I. Guerrero,² John Kupelian,¹ Simon Doss-Gollin,³ Kinga K. Smolen,^{3,4} Simon D. van Haren,^{3,4} Philippe Armand,^{2,4} Ofer Levy,^{3,4,9} David R. Walt,^{4,5,6} Lindsey R. Baden,^{1,2,4,b} and Nicolas C. Issa^{1,2,4,b}

¹Division of Infectious Diseases, Brigham and Women's Hospital, Boston, Massachusetts, USA, ²Dana-Farber Cancer Institute, Boston, Massachusetts, USA, ³Precision Vaccines Program, Division of Infectious Diseases, Boston Children's Hospital, Boston, Massachusetts, USA, ⁴Harvard Medical School, Boston, Massachusetts, USA, ⁵Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts, USA, ⁶Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts, USA, ⁷School of Pharmacy, National Taiwan University, Taipei, Taiwan, ⁸Division of Infectious Diseases, Centre Hospitalier de l'Université de Montréal, Montreal, Quebec, Canada, and ⁹Broad Institute of MIT & Harvard, Cambridge, Massachusetts, USA

Background. Patients with lymphoid malignancies are at risk for poor coronavirus disease 2019 (COVID-19)-related outcomes and have reduced vaccine-induced immune responses. Currently, a 3-dose primary regimen of mRNA vaccines is recommended in the United States for immunocompromised hosts.

Methods. A prospective cohort study of healthy adults ($n=27$) and patients with lymphoid malignancies ($n=94$) was conducted, with longitudinal follow-up through completion of a 2- or 3-dose primary mRNA COVID vaccine series, respectively. Humoral responses were assessed in all participants, and cellular immunity was assessed in a subset of participants.

Results. The rate of seroconversion (68.1% vs 100%) and the magnitude of peak anti-S immunoglobulin G (IgG) titer (median anti-S IgG = 32.4, IQR = 0.48–75.0 vs median anti-S IgG = 72.6, IQR 51.1–100.1; $P = .0202$) were both significantly lower in patients with lymphoid malignancies compared to the healthy cohort. However, peak titers of patients with lymphoid malignancies who responded to vaccination were similar to healthy cohort titers (median anti-S IgG = 64.3; IQR, 23.7–161.5; $P = .7424$). The third dose seroconverted 7 of 41 (17.1%) patients who were seronegative after the first 2 doses. Although most patients with lymphoid malignancies produced vaccine-induced T-cell responses in the subset studied, B-cell frequencies were low with minimal memory cell formation.

Conclusions. A 3-dose primary mRNA series enhanced anti-S IgG responses to titers equivalent to healthy adults in patients with lymphoid malignancies who were seropositive after the first 2 doses and seroconverted 17.1% who were seronegative after the first 2 doses. T-cell responses were present, raising the possibility that the vaccines may confer some cell-based protection even if not measurable by anti-S IgG.

Keywords. lymphoid malignancies; mRNA vaccines; primary series; SARS-CoV-2.

Although two nanoparticle-encapsulated mRNA severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines—mRNA-1273 (Moderna, Inc.) and BNT162b2 (Pfizer BioNTech, Inc.)—have demonstrated high efficacy and safety for the general population [1, 2], the humoral and cellular

kinetics in patients with lymphoid malignancies have not been well described after a primary series (three doses). We have previously described the humoral response after two doses in patients with lymphoid malignancies [3], which is consistent with additional studies of decreased immunogenicity in these patients [4, 5]. Underlying type of lymphoid malignancy and type and timing of therapy also impact SARS-CoV-2 antibody responses in these patients [6]. In particular, patients with lymphoid malignancies who have received specific therapies—such as anti-CD20 agents, Bruton tyrosine kinase (BTK) inhibitors, CD19-directed chimeric antigen receptor therapy (CAR-T), or stem cell transplantation—appear to have impaired humoral responses [7–9], although they may have some degree of cellular immunity [10, 11]. The degree of protection based on cellular immunity alone is not well described. Based on the existing data of poor immunogenicity in immunocompromised hosts with a two-dose mRNA series, the US Food and Drug Administration (FDA) amended the Emergency Use

Received 04 May 2022; editorial decision 10 August 2022; accepted 11 August 2022; published online 16 August 2022

^aA. C. S. and J. L. C. are cofirst authors.

^bL. R. B. and N. C. I. are cosenior authors.

Correspondence: A. Sherman, MD, Brigham and Women's Hospital, 15 Francis Street, Boston, MA 02115 (acs Sherman@bwh.harvard.edu).

Open Forum Infectious Diseases®

© The Author(s) 2022. Published by Oxford University Press on behalf of Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<https://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
<https://doi.org/10.1093/ofid/ofac417>

Authorization (EUA) to recommend a three-dose mRNA vaccine series (mRNA-1273 or BNT162b2) for immunocompromised hosts in August 2021 [12], which is now considered a primary series for this population. Few studies have evaluated the immunogenicity effects of a third dose for patients with hematological malignancies [13, 14], although data from a prospective registry (The Leukemia & Lymphoma Society National Registry, ClinicalTrials.gov Identifier NCT04794387) demonstrate that a third dose may produce a humoral response in a proportion of patients who had a suboptimal response to the first two doses [6].

There is an urgent need to better understand vaccine-induced immunogenicity in the context of heterogeneous host characteristics to improve protection for these patients who are at higher risk for poor outcomes from coronavirus disease 2019 (COVID-19). SARS-CoV-2 monoclonal antibody infusions have been a temporary solution for prevention, although this is a limited resource with short-lived efficacy due to immune escape mechanisms [15]. With limited tools in our current arsenal to protect immunocompromised patients against SARS-CoV-2, additional boosters have been approved [16], although these recommendations are based on imperfect data on a diverse range of immunocompromised hosts (eg, solid organ transplant recipients), which may not reflect the same immunodeficient characteristics as those with lymphoid malignancies. In addition, patients with lymphoid malignancies, and especially those receiving B cell-depleting therapies, often experience prolonged courses of SARS-CoV-2 infection that may play a key role in the generation of variant strains [17–19]. Thus, to generate optimal prevention strategies, the humoral and cellular aspects of immunity in this population and the nuanced effects of host characteristics and anticancer treatments must be better elucidated. In this study, we describe the SARS-CoV-2-specific immune humoral and cellular responses in a cohort of patients with lymphoid malignancies who received a primary series of three doses of mRNA COVID-19 vaccines. In the absence of a known correlate of protection, we compared these responses to the immunogenicity of a primary series (two doses of mRNA vaccines) in a cohort of healthy adults to further characterize the immune response.

METHODS

Study Population and Specimen Collection

A prospective cohort study was conducted at Brigham and Women's Hospital (BWH) and Dana-Farber Cancer Institute (DFCI) from December 10, 2020 to February 15, 2022 with approval by the BWH and DFCI Institutional Review Board. All participants provided written informed consent, with eligibility and additional details in the [Supplementary Materials](#). Participants were excluded at the time of enrollment if they

had a prior history of COVID-19 or had received SARS-CoV-2 monoclonal antibodies. For the **lymphoid malignancy cohort 1**, patients were enrolled before first dose, with blood drawn at Day 0 (prevaccination), Day 21–28 (at the time of the second dose), Day 56, Day 112, and Day 196 as well as pre-third dose (up to 7 days before the third dose), 21–28 days after the third dose, and up to 3 months after the third dose. To increase the sample size, a separate cohort of patients (**lymphoid malignancy cohort 2**) were enrolled after the third vaccine dose was approved for immunocompromised individuals. In alignment with vaccination campaigns that were launched after the third dose approval, blood was collected at clinical satellite sites on the day of the third dose, 21–28 days after the third dose, and up to 3 months after the third dose. For the healthy cohort, blood was drawn at Day 0 (prevaccination), Day 21–28 (at the time of the second dose), Day 56, Day 112, and Day 196.

Serologic Assays: Simoa and Roche Elecsys Anti-Sars-CoV-2 S

The multiplexed, single-molecule array (Simoa) assay, quantitative detection of immunoglobulin G (IgG) antibodies against the Spike (S) protein and nucleocapsid (N) proteins were assessed for lymphoid malignancy cohort 1, with methods described previously [3, 20, 21]. The Simoa assay has a positivity threshold of 1.07 and 5.2 normalized average enzymes per bead (AEB) for anti-S IgG and anti-N IgG, respectively. The Roche Elecsys Anti-SARS-CoV-2 S assay, an electrochemiluminescence immunoassay, was used to analyze serum samples on the cobas c602 immunochemistry module in the lymphoid malignancy cohort 2 to assess total anti-S IgG [22]. The positive threshold is a cutoff index of ≥ 0.8 U/mL with an upper limit of 2500 U/mL. The samples from the healthy cohort were evaluated using both the Simoa and Roche assays. In this study, a “responder” was defined by any positive anti-S titer during the study period, according to the cutoff values for the given assay.

Cellular Immunity: Activation Induced Marker Assay

The activation induced marker (AIM) assay was adapted from protocols previously described [23, 24]. Flow cytometry was used to phenotype B and T cells after stimulation in the AIM assay. Fluorescent intensities were acquired using a LSRFortessa flow cytometer (Becton Dickinson) and data were analyzed using FlowJo software version 10. Additional details are in the [Supplementary Materials](#).

Statistical Analyses

Median (interquartile range [IQR]) and Mann-Whitney *U* test were used to compare continuous variables. Count (percentage) and Fisher's exact test were used to compare categorical variables. Correlation between months from CD20 therapy and anti-S IgG titer was evaluated using linear regression.

Two-sided *P* values less than 0.05 were considered statistically significant. All analyses were conducted in R version 4.1.2 (<https://www.R-project.org/>).

RESULTS

Demographics and Disease Characteristics

Overall, 27 healthy participants and 103 patients with lymphoid malignancy were enrolled. Eight patients with lymphoid malignancy were excluded from the analysis due to receipt of non-mRNA COVID-19 vaccines during the study period, and 1 patient was excluded because he/she elected to terminate the study early. Therefore, a total of 94 participants were included in the analysis, with 41 participants in lymphoid malignancy cohort 1 and 53 participants in lymphoid malignancy cohort 2.

In the healthy cohort, 15 (56.0%) patients were female, and the median age 24.3 (IQR, 22.8–28.7), with 11 (40.7%) receiving 2 BNT162b2 (30 mcg) doses and 16 (59.3%) receiving 2 mRNA-1273 (100 mcg) doses. Only 1 participant in the healthy cohort had COVID-19 infection during the study period, with both a reported history and a positive anti-N IgG titer.

In the lymphoid malignancy combined cohort ($n=94$), 49 (52.1%) were female, and the median age 65.5 was years (IQR, 57.5–72.8), with 56 (59.6) receiving 3 BNT162b2 (30 mcg) doses, 26 (27.7%) receiving 3 mRNA-1273 (100 mcg) doses, 2 (2.1%) receiving 2 BNT162b2 doses and 1 mRNA-1273 dose, and 7 (7.4%) receiving 2 mRNA-1273 doses and 1 BNT162b2 dose (Table 1). Three patients did not receive a third dose of mRNA vaccine due to clinician discretion, and 6 patients were not able to return to clinic for a post third dose blood draw. Most patients had an underlying diagnosis of chronic lymphocytic leukemia (CLL) (59, 62.8%), with 9 other lymphoma types represented (Table 1). The treatment status, defined as treatment naive, history of prior treatment (before the first vaccine dose), or active treatment (received treatment at any time after the first vaccine dose) was split among participants, 22 (23.4%), 27 (28.7%), 45 (47.9%), respectively, with specific treatments further characterized in Table 1.

Severe Acute Respiratory Syndrome Coronavirus 2 Infection During the Study Period

Eleven participants in the lymphoma cohorts and one participant in the healthy cohort developed COVID-19 infection during the study period, as determined by reported history, positive SARS-CoV-2 polymerase chain reaction in the electronic medical record, or positive anti-N IgG titer (Supplementary Table 1). Seven of the eleven lymphoma patients had a positive anti-S response to vaccination before infection, one seroconverted after infection, and two participants had no anti-S response before or after infection. One participant had no earlier titers (in the lymphoid group 2 cohort),

and thus it is unknown whether post third dose titers were positive from infection or vaccination or both. The participant in the healthy cohort developed COVID-19 seven months after completing the two-dose vaccine series.

Seroconversion After Vaccination

All participants in the healthy cohort (27 of 27) had a positive response after the first mRNA COVID vaccine dose, which was maintained for the 6-month follow-up period, with modest antibody waning over time that was still maintained well above the positivity threshold (Figure 1). In contrast, there was a combined 68.1% seropositivity for patients with lymphoid malignancies (Supplementary Table 2, with breakdown of seropositivity by patient characteristics) after a three-dose series. In the lymphoid malignancy cohort 1, 14 of 41 (34.1%) had a positive anti-S titer after the first dose, 22 of 41 (53.7%) were positive after the second dose, and 22 of 32 (68.8%) were positive after the third dose. Patients did not necessarily maintain a positive response over time, with evidence of seroreversion. Only two participants who were seronegative after the first two doses had seroconversion after the third dose, with low titers (median anti-IgG = 6.55 nAEB; IQR, 3.82–9.28). Of the 22 of 53 participants in lymphoid malignancy cohort 2 who were seronegative before receipt of the third dose, only five seroconverted after receiving a third dose, with an overall low median titer of 123.5 U/mL (IQR, 100.9–357.7).

Anti-S Immunoglobulin G Responses After First and Second Doses

There was greater variability in the lymphoid malignancy cohort 1 after the first and second doses compared to the healthy controls (Figure 2A). At 21–28 days post first dose, the median anti-S IgG titer was 25.5 (IQR, 14.4–38.1) compared to 0.1 (IQR, 0.1–1.6) in the healthy cohort and the lymphoid malignancy cohort 1, respectively ($P < .001$), with significantly lower titers at days 49–56 (post second dose) and days 168–250 as well (Table 2). Even among the responders ($n=28$) with lymphoid malignancies (Figure 2B), the magnitude of response was significantly lower at 21–28 days (median anti-S IgG = 0.8; IQR, 0.1–8.5), 49–56 days (post second dose; median anti-S IgG = 27.2; IQR, 9.4–49.5), and 168–250 days (anti-S IgG = 2.4; IQR, 0.6–11.7) compared to the healthy cohort (median anti-S IgG = 25.5 and IQR = 14.4–38.1, median anti-S IgG = 70.3 and IQR = 49.7–95.8, and median anti-S IgG = 17.1 and IQR = 10.4–25.0, respectively) (Table 2).

Anti-S Immunoglobulin G Responses After a Third Dose

After the third dose (median 45 days; IQR, 25–132.5), the median anti-S IgG for the entire lymphoid malignancy cohort 1 (median anti-S IgG = 32.4; IQR, 0.48–75.0) and the responders (median anti-S IgG = 64.3; IQR, 23.7–161.5) was compared to the healthy cohort 28 days after a 2-dose series completion (median anti-S IgG = 72.6; IQR, 51.1–100.1) (Table 2).

Table 1. Demographics and Disease and Treatment Characteristics

Characteristics	Healthy Cohort (n=27)	Lymphoid Malignancy Cohort 1 (n=41)	Lymphoid Malignancy Cohort 2 (n=53)	Combined Lymphoid Malignancy (n=94)
Female sex, n (%)	15 (56)	23 (52.3)	26 (49.1)	49 (52.1)
Age at vaccine dose 1, median (IQR)	24.3 (22.8–28.7)	68.4 (60.3–73.1)	63.0 (56.8–70.6)	65.5 (57.5–72.8)
Vaccine type ^a (first/second/third dose) n (%)				
Pfizer/Pfizer/Pfizer	n/a	22 (53.7)	34 (64.2)	56 (59.6)
Moderna/Moderna/Moderna	n/a	11 (26.8)	15 (28.3)	26 (27.7)
Pfizer/Pfizer/Moderna	n/a	2 (4.9)	0 (0)	2 (2.1)
Moderna/Moderna/Pfizer	n/a	3 (7.3)	4 (7.5)	7 (7.4)
Pfizer/Pfizer	11 (40.7)	2 (4.9)	n/a	2 (2.1)
Moderna/Moderna	16 (59.3)	1 (2.4)	n/a	1 (1.1)
Median time between doses, days (IQR)				
Between 1st and 2nd doses	28 (21–28)	21 (21–27)	22 (21–28)	21 (21–28)
Between 2nd and 3rd doses	n/a	168 (146–195)	167 (152–185)	168 (149–186)
History of COVID-19 infection during study period (reported or positive anti-N), n (%)	1 (3.7)	5 (12.2)	6 (11.3)	10 (10.6)
Median time (days) from third dose to postthird dose titer, (IQR)	n/a	45 (25.0–132.5)	27 (23–29)	28 (24–35)
Disease, n (%)				
CLL	n/a	23 (56.1)	36 (64.3)	59 (62.8)
DLBCL	n/a	2 (4.9)	3 (5.7)	5 (5.3)
MCL	n/a	5 (11.6)	2 (3.8)	7 (7.4)
FL	n/a	3 (7.3)	4 (7.5)	7 (7.4)
MZL	n/a	2 (4.7)	2 (3.8)	4 (4.3)
HL	n/a	4 (9.3)	1 (1.9)	5 (5.3)
Angioimmunoblastic T-cell lymphoma	n/a	1 (2.4)	0	1 (1.1)
CNS Lymphoma	n/a	1 (2.4)	0	1 (1.1)
PLL	n/a	0	1 (1.9)	1 (1.1)
T-LGL	n/a	0	1 (1.9)	1 (1.1)
Treatment Status, n (%)				
Treatment naive	n/a	9 (21.9)	13 (23.2)	22 (23.4)
Prior treatment	n/a	12 (29.3)	15 (26.8)	27 (28.7)
Active treatment	n/a	20 (48.8)	25 (44.6)	45 (47.9)
Treatment Groups, n (%)				
Observation	n/a	9 (21.9)	13 (24.5)	22 (23.4)
BTKI ^b	n/a	10 (24.3)	10 (18.9)	20 (21.3)
Venetoclax monotherapy	n/a	5 (12.2)	3 (5.7)	8 (8.5)
CD20 Antibody therapy ^c	n/a	24 (58.5)	29 (54.7)	53 (56.4)
CD20 Ab within 12 months of 1st dose, n (%)	n/a	11/24 (45.8)	7/29 (24.1)	18/53 (34.0)
CD20 Ab beyond 12 months of 1st dose, n (%)	n/a	13/24 (54.2)	22/29 (75.9)	35/53 (66.0)
CD20 monotherapy	n/a	7 (17.1)	8 (15.1)	15 (16.0)
CD20 + BTKI	n/a	6 (14.6)	4 (7.5)	10 (10.6)
CD20 + venetoclax	n/a	5 (12.2)	5 (9.4)	10 (10.6)
CD20 + chemotherapy	n/a	1 (2.4)	11 (20.8)	12 (12.8)
CD20 + autologous stem cell transplant	n/a	2 (4.9)	0	2 (2.1)
CD20 + CAR-T cell therapy	n/a	3 (7.3)	1 (1.9)	4 (4.3)
Chemotherapy	n/a	2 (4.9)	3 (5.7)	5 (5.3)
Chemotherapy + autologous stem cell transplant	n/a	1 (2.4)	0	1 (1.1)
Autologous stem cell transplant	n/a	3 (7.3)	0	3 (3.2)
CAR-T cell therapy	n/a	3 (7.3)	1 (1.9)	4 (4.3)
Median number of prior lines of therapy (IQR)	n/a	2 (1–3)	1 (1–1)	1 (1–3)
IVIg during study period, n (%)	n/a	2 (4.8)	6 (10.7)	8 (8.5)
Median baseline IgG, mg/dL (IQR)	n/a	633 (483–937)	885 (708–969)	646 (489–938)

Abbreviations: Ab, antibody; BTKI, Bruton tyrosine kinase inhibitors; CAR-T, chimeric antigen receptor therapy; CLL, chronic lymphocytic leukemia; CNS, central nervous system; COVID-19, coronavirus disease 2019; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HL, Hodgkin's lymphoma; IgG, immunoglobulin G; IQR, interquartile range; IVIG, intravenous immunoglobulin; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; n/a, not applicable; PLL, prolymphocytic leukemia; T-LGL, T-cell large granular lymphocytic leukemia;

^a100 mcg per dose for the Moderna vaccine and 30 mcg per dose for the Pfizer vaccine.

^bBruton tyrosine kinase inhibitors: ibrutinib, acalabrutinib, Zanabrutinib, pirtobrutinib (LOXO-305).

^cCD20 antibody therapy includes the following: rituximab, Obinutuzumab. CD20 antibodies could be used as monotherapy or in combination with chemotherapy or targeted therapy.

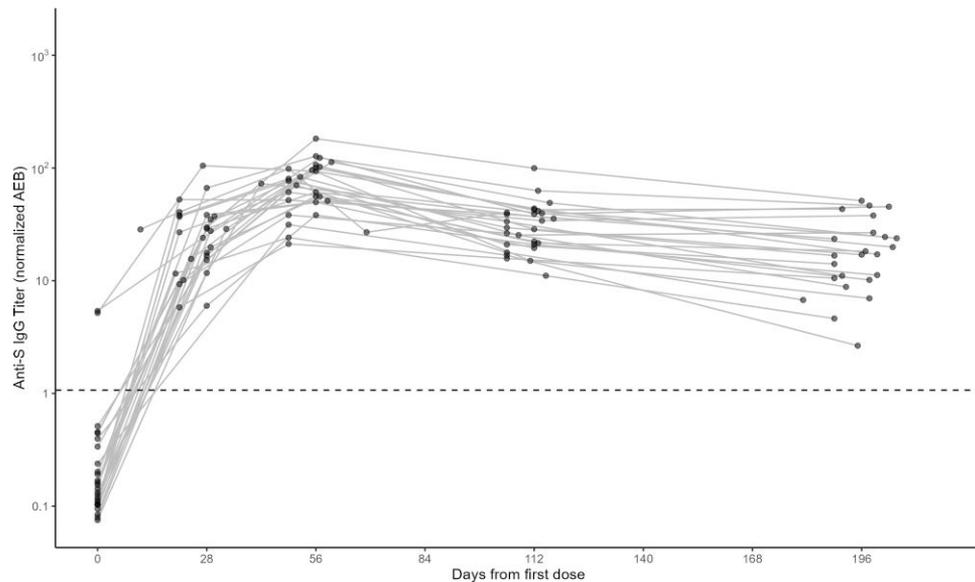


Figure 1. Anti-S IgG titers from the time of first vaccine dose (day 0) to 6 months after series completion (through day 205) in healthy participants ($n = 27$). The dotted line at 1.07 represents the positivity threshold for the Simoa assay.

The median titer was significantly lower in lymphoid malignancy cohort 1 ($P = .0202$) but not significantly different for the responders ($P = .7424$), indicating that the responders were able to achieve similar titer levels to the healthy cohort after the third dose.

In the lymphoid malignancy cohort 2, anti-S total IgG titers after the third dose were collected clinically using the Roche assay. The median time from the anti-S IgG titer from the third dose was 27 days (IQR, 23–29), with a median anti-S IgG titer of 670 U/mL (IQR, 0–2500) for all patients and 2500 U/mL (IQR, 592–2500) for the responders. In comparison, the median anti-S IgG titer in the healthy cohort was 2500 U/mL (IQR, 1933.5–2500) at 28 days after a 2-dose series completion, which is significantly higher compared to the entire cohort 2 ($P = .0032$) but not significantly different compared to the responders only ($P = .4574$) (Supplementary Table 3), similar to the results described for cohort 1.

Responders by Treatment in the Lymphoid Malignancy Cohorts

Patients who were treatment naive had a higher rate of response (95.5%) compared to those who had received prior treatment (70.4%) or were actively receiving therapies (53.3%) during the study period. Within the treatment groups, participants who received prior or active venetoclax monotherapy, CAR-T therapy, CD20 antibody monotherapy, or combination of venetoclax and CD20 therapy had the lowest rates of response (50.0%, 50.0%, 40.0%, and 30.0%, respectively). There was a positive although nonsignificant correlation between months from CD20 therapy and anti-S IgG titer ($r^2 = 0.1035$, $P = .125$) (Supplementary Figure 1A), in which the highest

anti-S IgG titer was selected for each participant who had received any CD20 therapy in the past, with a stronger linear correlation observed in participants who had received CD20 monotherapy only ($r^2 = 0.58$, $P = .0454$) (Supplementary Figure 1B).

B- and T-Cell Immunophenotyping Results

A subset of the overall lymphoid malignancy cohort (Supplementary Table 4) opted to have additional blood drawn for cellular analyses ($n = 17$) up to the third dose. Quantification of circulating S protein-specific B cells by flow cytometry confirmed that vaccine-induced B cells could be observed in some of the participants after the first and second immunizations (Figure 3A). Phenotypic analysis of these B cells shows that the absence in increased B-cell frequency coincides with an impairment in class-switch recombination and memory formation, as indicated by the absence of vaccine-specific IgD-CD27⁺ B cells (Supplementary Figure 2A). In contrast to the frequency of vaccine-induced B cells, an appreciable increase in vaccine-induced CD4⁺ and, in particular, CD8⁺ T cells was observed after the first and second immunization (Figure 3B and C). Phenotypic analysis reveals that vaccine-specific T cells were of antigen-experienced phenotype (CD62L-negative) and capable of producing interleukin (IL)-2 and interferon (IFN)- γ after stimulation with antigenic peptides (Supplementary Figure 2B and C). In the cellular subset, 9 of 17 (52.9%) had a positive anti-S IgG response (Supplementary Table 4). It is interesting to note that among the eight participants in the cellular subset who had no anti-S IgG response, seven had vaccine-induced CD4⁺ and CD8⁺ responses and produced IL-2

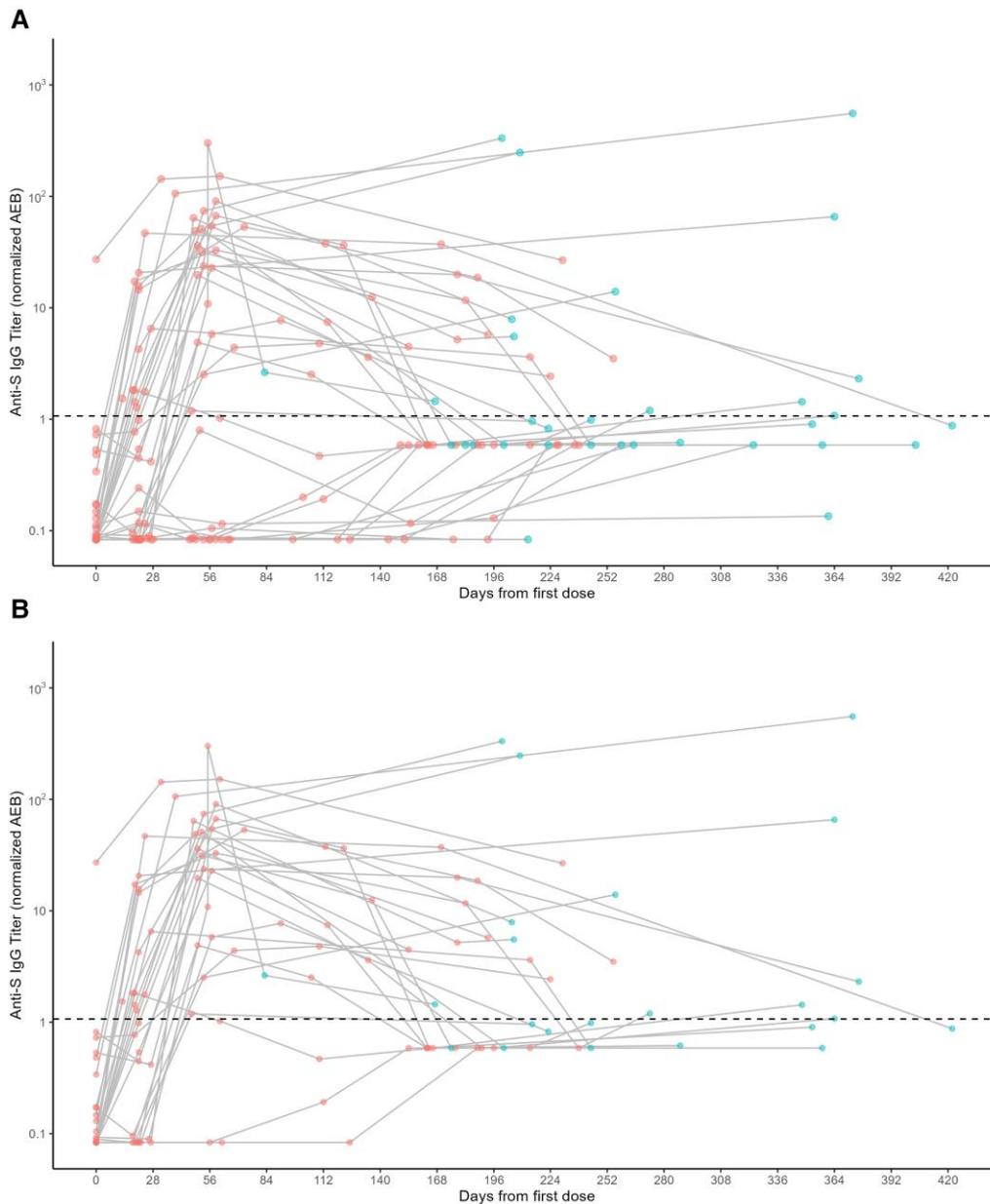


Figure 2. Anti-S IgG titers from the time of first vaccine dose (day 0) to day 420 in all participants in the lymphoid malignancy cohort 1 ($n = 41$; *A*) and only the responders ($n = 28$) in lymphoid malignancy cohort 1 (*B*). Pink dots represent titers drawn from day 0 through post second dose titers; blue dots represent titers drawn after the third vaccine dose. The dotted line at 1.07 represents the positivity threshold for the Simoa assay.

and IFN- γ after stimulation with antigenic peptides. Both B- and T-cell frequencies waned over time.

DISCUSSION

In this study, we describe the kinetics of anti-S IgG response through a three-dose primary mRNA series in patients with lymphoid malignancies and found a decreased median humoral response compared to a two-dose primary mRNA series in healthy individuals, with only 68.1% patients with lymphoid

malignancy seroconverting at any point during the study period. Although others have also described decreased seroconversion in patients with lymphoid malignancies after SARS-CoV-2 vaccination [14], our study is unique in evaluating the humoral responses longitudinally through a third dose and comparing to a healthy cohort, as well as in evaluating cellular responses over time in a highly characterized cohort.

Seroconversion rates alone do not fully capture the quantity and quality of the humoral response nor timing of seroconversion, all important aspects of vaccine immunogenicity. In our study, only a

Table 2. Median Anti-S IgG Titers Over Time in Healthy Adults vs Lymphoid Malignancy Cohort 1

Simoa Assay (Normalized AEB)	Healthy Cohort (n = 27)	Lymphoid Malignancy Cohort 1 (n = 41)	Cohort 1: Responders Only (n = 28)	P Value (Healthy vs Cohort 1)	P Value (Healthy vs Responders Only)
21–28 days postfirst dose, median (IQR)	25.5 (14.4–38.1)	0.1 (0.1–1.6)	0.8 (0.1–8.5)	<i>P</i> < .001	<i>P</i> < .001
49–56 days postfirst dose, median (IQR)	70.3 (49.7–95.8)	15.3 (0.6–39.4)	27.2 (9.4–49.5)	<i>P</i> < .001	<i>P</i> < .001
168–250 days postfirst dose, median (IQR)	17.1 (10.4–25.0)	0.6 (0.6–5.6)	2.4 (0.6–11.7)	<i>P</i> < .001	<i>P</i> < .001
Postseries completion ^a , median (IQR)	72.6 (51.1–100.1)	32.4 (0.48–75.0)	64.3 (23.7–161.5)	<i>P</i> = .0202	<i>P</i> = .7424

Abbreviations: AEB, average enzymes per bead; IgG, immunoglobulin G; IQR, interquartile range.

^aPostseries completion titers were taken ~1–2 months after the 2-dose series (healthy cohort) or 3-dose primary series (lymphoid malignancy cohort).

small number of patients with lymphoid malignancy who did not respond to the first two doses seroconverted after a third dose. Although this may still support the use of a third dose to increase the number of responders, those who seroconverted after the third dose had low titers, which may not confer strong protection. Furthermore, a positive antibody response at one point in time does not predict a sustained response in this population, as seen for the participants in our study who had waning antibody over time. Possible explanations may include lack of memory B-cell formation, rapid waning of antibody, and/or specific immunosuppressive therapies given after the vaccine dose.

However, among participants in the lymphoid malignancy cohort who seroconverted after the first 2 doses, 3 doses were needed to achieve similar titer levels to those in the healthy cohort post series, supporting the use of a third dose for this subset of patients. Although correlates of protection for SARS-CoV-2 vaccines have not yet been clearly defined [25], the comparison of titer levels to the healthy controls offers more insight to the degree of protection (rather than seroconversion alone) based on available evidence from phase 3 trials [26].

With respect to cellular immunity, some participants demonstrated an increased frequency of S-positive B cells after vaccination although generally low, which is not unexpected given B cell-directed therapies. However, most demonstrated increased frequencies of vaccine-induced CD4⁺ and CD8⁺ cells, consistent with other studies of immunocompromised hosts [13, 27]. We found that among participants in the cellular subset who had no humoral response, most were able to produce vaccine-induced CD4⁺ and CD8⁺ responses. Thus, patients with lymphoid malignancy receiving mRNA vaccines may have partial protection due to cellular immunity, even in the absence of a humoral response. However, the durability of this response could be short lived, with decreasing frequencies in the periphery observed for both B- and T-cells at 5 to 6 months post second doses of vaccine. Alternatively, the decrease in peripheral frequency could be the result of homing to tissue or lymph node, thus potentially improving durability, if T-cells have a high CCR7 expression [28, 29]. Further studies

should evaluate whether additional vaccine doses can bolster cellular immunity or induce a more durable T-cell response and determine how cellular immunity correlates with clinical efficacy against severe COVID-19.

Limitations of this study include the relatively small sample size, precluding assessment of potential differences in humoral or cellular responses based on age, vaccine type, disease characteristics, or treatments. However, we were able to demonstrate important trends by disease type and treatments, which should be further explored in larger studies. Although we used two different assays to measure anti-S IgG response, we have previously demonstrated high concordance between the Simoa and Roche assays [9]. Future studies should address the clinical efficacy and the role of both humoral (including neutralization) and cellular immunity in conferring protection against COVID-19 in patients with lymphoid malignancies. Due to the timing of our study, third doses for immunocompromised hosts were not available until approximately six months after the first two mRNA vaccine doses were administered. Thus, in our cohort, the median time between the second and third dose was 168 days (IQR, 146–195). The Centers for Disease Control and Prevention now recommends that immunocompromised hosts may receive the third dose of the primary series as early as one month after the second dose [30]. Our study does not assess how the timing of the third dose may affect antibody response, nor how additional boosters (fourth or fifth doses) may affect immunity; these variables will be important to assess for future studies.

CONCLUSIONS

In summary, although a primary series of three doses of mRNA vaccines is currently recommended in the United States for immunocompromised hosts, our study demonstrates that the third dose only bolsters humoral responses in those who could produce an endogenous response to one or two doses, with a similar magnitude of titer compared to healthy individuals after a primary series. However, as has been described in solid organ transplant recipients [31], a third dose did not induce substantial seroconversion among those who were seronegative after the first 2 doses, nor did it

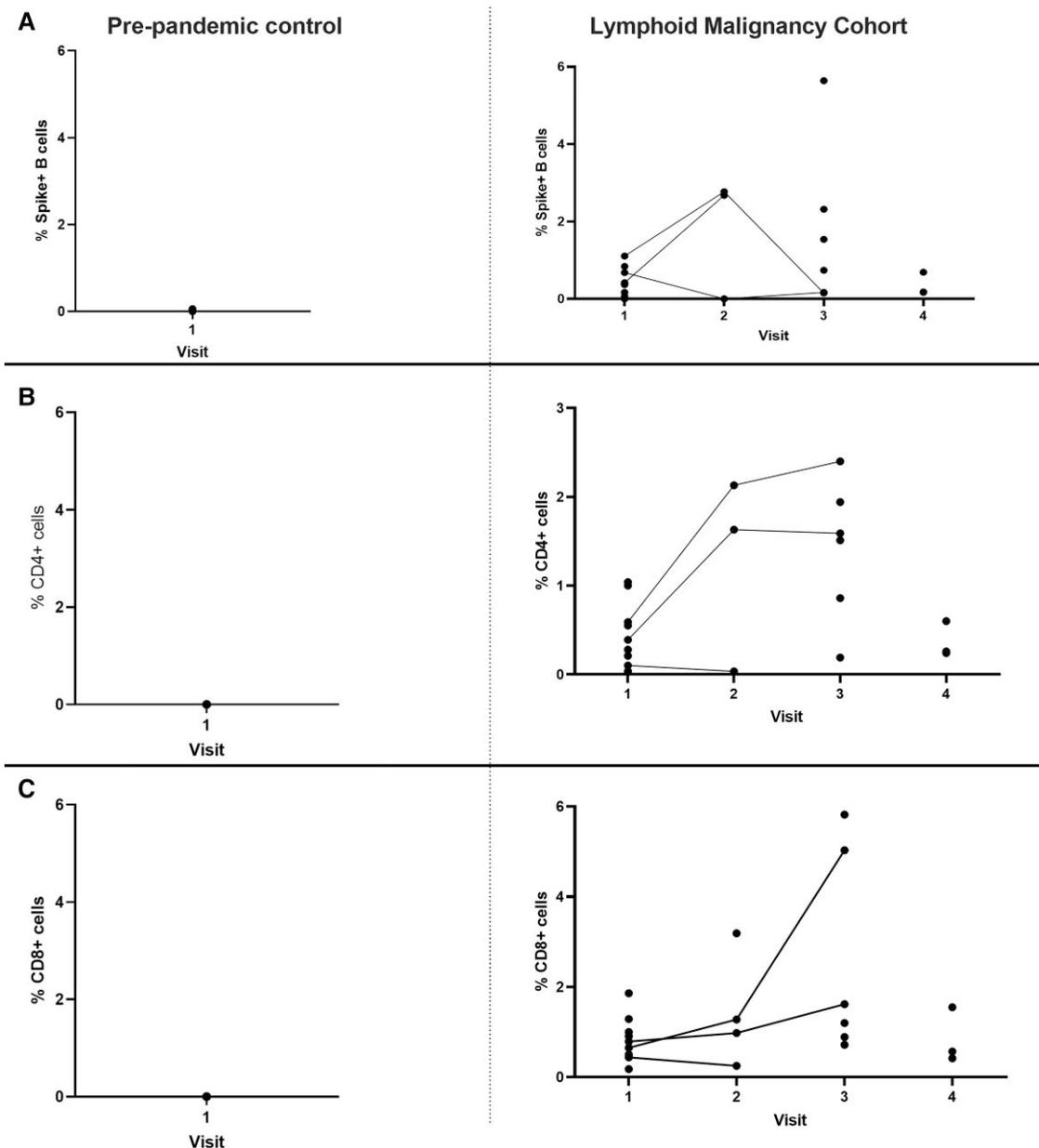


Figure 3. The percentage frequency of Spike-specific B cells (A), CD4⁺ cells (B), and CD8⁺ cells (C) are shown. A subset of participants from both lymphoid malignancy cohort 1 and 2 ($n=17$) had cellular responses assessed at baseline (the time of dose 1; Visit 1), 21–28 days later (the time of dose 2; Visit 2), 49–56 days later (1 month after dose 2; Visit 3), and 5–6 months later (the time of dose 3; Visit 4). Prepandemic samples were run as controls and shown on the left.

dependably convert those who had seroreverted. In addition, T-cell immunity was induced, even in those without a humoral response. Thus, measuring antibody responses alone may not be a full measure of SARS-CoV-2 protection.

Given the increasing appreciation of the variation in immunity between individuals, we are entering an era of precision vaccinology [32]. In this context, future vaccination recommendations must consider specific host characteristics of patients with lymphoid malignancies, avoiding a classification of this subpopulation under a generic umbrella of “immuno-compromised hosts.” Precision immunization strategies to

protect this vulnerable population according to disease, prior anticancer therapeutics, and other host characteristics are urgently needed to provide improved protection and reduce prolonged SARS-CoV-2 shedding, thereby providing broader public health benefits by curbing the pandemic and potentially reducing generation of variant strains.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyrighted and are the sole responsibility of the

authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

We thank Natalie Izaguirre, Bruce Bausk, Urwah Kanwal, Megan Powell, Joseph Larson, and Peter Baker for their assistance with clinical management, including organization of phlebotomy, data entry, and follow-up visits. Xiaofang Li and Salena Von assisted with processing of samples. O. L. thanks Boston Children's Hospital President and Chief Operating Officer Dr. Kevin Churchwell and Physician-in-Chief Dr. Gary Fleisher for support of the Precision Vaccines Program.

Financial support. D. R. W., A. C. S, and L. R. B. received financial support for the present work from Barbara and Amos Hostetter and from the Check Foundation. L. R. B. received support from Harvard Clinical and Translational Science Center (Grant Number 1UL1TR002541-01), from the National Center for Advancing Translational Science. O. L. and the Precision Vaccines Program were supported in part via a Massachusetts Coalition for Pathogen Readiness (Mass-CPR) award via Harvard Medical School, the Boston Children's Hospital Department of Pediatrics, and philanthropy from the Boston Investment Council and the Barry family.

Potential conflicts of interest. D. R. W. has a financial interest in Quanterix, a company developing an ultrasensitive digital immunoassay platform; he is an inventor of the Simoa technology, a founder of the company, and a member of its board of directors. D. R. W.'s interests were reviewed and are managed by BWH and Partners HealthCare in accordance with their conflict-of-interest policies. O. L. is a named inventor on patents relating to human in vitro systems modeling vaccine action and vaccine adjuvants. J. L. C. receives consulting fees from Incyte and Karyopharm. J. L. C. reports grant support, paid to her institution from AbbVie and Bayer. A. P. receives research funds from Merck, BMS, Affimed, Adaptive, Roche, Tensha, Otsuka, Sigma Tau, Genentech, IGM, and Kite. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med* **2021**; 384:403–16.
2. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. *N Engl J Med* **2020**; 383:2603–15.
3. Crombie JL, Sherman AC, Cheng CA, et al. Activity of mRNA COVID-19 vaccines in patients with lymphoid malignancies. *Blood Adv* **2021**; 5:3062–5.
4. Herishanu Y, Avivi I, Aharon A, et al. Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. *Blood* **2021**; 137:3165–73.
5. Roeker LE, Knorr DA, Thompson MC, et al. COVID-19 vaccine efficacy in patients with chronic lymphocytic leukemia. *Leukemia* **2021**; 35:2703–5.
6. Greenberger LM, Saltzman LA, Senefeld JW, Johnson PW, DeGennaro LJ, Nichols GL. Anti-spike antibody response to SARS-CoV-2 booster vaccination in patients with B cell-derived hematologic malignancies. *Cancer Cell* **2021**; 39: 1297–9.
7. Marchesi F, Pimpinelli F, Giannarelli D, et al. Impact of anti-CD20 monoclonal antibodies on serologic response to BNT162b2 vaccine in B-cell non-Hodgkin's lymphomas. *Leukemia* **2022**; 36:588–90.
8. Parry H, McIlroy G, Bruton R, et al. Antibody responses after first and second COVID-19 vaccination in patients with chronic lymphocytic leukaemia. *Blood Cancer J* **2021**; 11:1–8.
9. Sherman AC, Desjardins M, Cheng CA, et al. SARS-CoV-2 mRNA vaccines in allogeneic hematopoietic stem cell transplant recipients: immunogenicity and reactivity. *Clin Infect Dis* **2021**:ciab930. doi: [10.1093/cid/ciab930](https://doi.org/10.1093/cid/ciab930).
10. Lyski ZL, Kim MS, Xthona Lee D, et al. Cellular and humoral immune response to mRNA COVID-19 vaccination in subjects with chronic lymphocytic leukemia. *Blood Adv* **2022**; 6:1207–11.
11. Malard F, Gaugler B, Gozlan J, et al. Weak immunogenicity of SARS-CoV-2 vaccine in patients with hematologic malignancies. *Blood Cancer J* **2021**; 11:1–8.
12. US Food and Drug Administration. Coronavirus (COVID-19) update: FDA authorizes additional vaccine dose for certain immunocompromised individuals. Available at: <https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-additional-vaccine-dose-certain-immunocompromised>. Accessed 24 October 2021.
13. Re D, Seitz-Polski B, Brglez V, et al. Humoral and cellular responses after a third dose of SARS-CoV-2 BNT162b2 vaccine in patients with lymphoid malignancies. *Nat Commun* **2022**; 13:864.
14. Herishanu Y, Rahav G, Levi S, et al. Efficacy of a third BNT162b2 mRNA COVID-19 vaccine dose in patients with CLL who failed standard 2-dose vaccination. *Blood* **2022**; 139:678–85.
15. Planas D, Saunders N, Maes P, et al. Considerable escape of SARS-CoV-2 omicron to antibody neutralization. *Nature* **2022**; 602:671–5.
16. US Food and Drug Administration. Coronavirus (COVID-19) update: FDA authorizes second booster dose of two COVID-19 vaccines for older and immunocompromised individuals. Available at: <https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-second-booster-dose-two-covid-19-vaccines-older-and-immunocompromised>. Accessed 31 March 2022.
17. Corey L, Beyrer C, Cohen MS, Michael NL, Bedford T, Rolland M. SARS-CoV-2 variants in patients with immunosuppression. *N Engl J Med* **2021**; 385:562–6.
18. Clark SA, Clark LE, Pan J, et al. SARS-CoV-2 evolution in an immunocompromised host reveals shared neutralization escape mechanisms. *Cell* **2021**; 184: 2605–17.e18.
19. Lee CY, Shah MK, Hoyos D, et al. Prolonged SARS-CoV-2 infection in patients with lymphoid malignancies. *Cancer Discov* **2022**; 12:62–73.
20. Norman M, Gilboa T, Ogata AF, et al. Ultrasensitive high-resolution profiling of early seroconversion in patients with COVID-19. *Nat Biomed Eng* **2020**; 4: 1180–7.
21. Ogata AF, Cheng CA, Desjardins M, et al. Circulating SARS-CoV-2 vaccine antigen detected in the plasma of mRNA-1273 vaccine recipients. *Clin Infect Dis* **2022**; 74:715–8.
22. Roche Diagnostics. Elecsys Anti-SARS-CoV-2. Available at: <https://www.fda.gov/media/137605/download>. Accessed 23 June 2021.
23. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* **2020**; 181:1489–501.e15.
24. Braun J, Loyal L, Frensch M, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. *Nature* **2020**; 587:270–4.
25. Sherman AC, Desjardins M, Baden LR. Vaccine-induced Severe Acute Respiratory Syndrome Coronavirus 2 antibody response and the path to accelerating development (determining a correlate of protection). *Clin Lab Med* **2022**; 42:111–28.
26. Gilbert PB, Montefiori DC, McDermott AB, et al. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science* **2022**; 375: 43–50.
27. Marasco V, Carniti C, Guidetti A, et al. T-cell immune response after mRNA SARS-CoV-2 vaccines is frequently detected also in the absence of seroconversion in patients with lymphoid malignancies. *Br J Haematol* **2022**; 196:548–58.
28. Neidleman J, Luo X, McGregor M, et al. mRNA vaccine-induced T cells respond identically to SARS-CoV-2 variants of concern but differ in longevity and homing properties depending on prior infection status. *Elife* **2021**; 10:e72619.
29. Jung JH, Rha MS, Sa M, et al. SARS-CoV-2-specific T cell memory is sustained in COVID-19 convalescent patients for 10 months with successful development of stem cell-like memory T cells. *Nat Commun* **2021**; 12:4043.
30. Centers for Disease Control and Prevention. COVID-19 vaccines for moderately or severely immunocompromised people. Available at: <https://www.cdc.gov/coronavirus/2019-ncov/vaccines/recommendations/immuno.html>. Accessed 20 March 2022.
31. Kamar N, Abravanel F, Marion O, Couat C, Izopet J, Del Bello A. Three doses of an mRNA COVID-19 vaccine in solid-organ transplant recipients. *N Engl J Med* **2021**; 385:661–2.
32. Soni D, Van Haren SD, Idoko OT, et al. Towards precision vaccines: lessons from the second international precision vaccines conference. *Front Immunol* **2020**; 11: 590373.