Humoral and cellular immunogenicity of SARS-CoV-2 vaccines in chronic lymphocytic leukemia: a prospective cohort study

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Key Points

- In this prospective study, 60% of patients with CLL developed SARS-CoV-2 antibodies and 80% developed functional T-cell responses after vaccination.

- Vaccinated patients with CLL developed antibodies against wild-type and variant SARS-CoV-2 viruses capable of binding and effector functions.

Chronic lymphocytic leukemia (CLL), the most common leukemia worldwide, is associated with increased COVID-19 mortality. Previous studies suggest only a portion of vaccinated CLL patients develop severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike antibodies. Whether the elicited antibodies are functional and/or accompanied by functional T-cell responses is unknown. This prospective cohort study included patients with CLL who received SARS-CoV-2 and PCV13 vaccines (not concurrently). The primary cohort included adults with CLL off therapy. Coprimary outcomes were serologic response to SARS-CoV-2 (receptor binding domain [RBD] immunoassay) and PCV13 vaccines (23-serotype IgG assay). Characterization of SARS-CoV-2 antibodies and their functional activity and assessment of functional T-cell responses was performed. Sixty percent (18/30) of patients demonstrated serologic responses to SARS-CoV-2 vaccination, appearing more frequent among treatment-naïve patients (72%). Among treatment-naïve patients, an absolute lymphocyte count ≤24 000/μL was associated with serologic response (94% vs 14%; $P < .001$). On interferon-γ release assays, 80% (16/20) of patients had functional spike-specific T-cell responses, including 78% (7/9) with a negative RBD immunoassay, a group enriched for prior B-cell–depleting therapies. A bead-based multiplex immunoassay identified antibodies against wild-type and variant SARS-CoV-2 (α, β, γ, and δ) in all tested patients and confirmed Fc-receptor binding and effector functions of these antibodies. Of 11 patients with negative RBD immunoassay after vaccination, 6 (55%) responded to an additional mRNA-based vaccine dose. The PCV13 serologic response rate was 29% (8/28). Our data demonstrate that SARS-CoV-2 vaccination induces functional T-cell and antibody responses in patients with CLL and provides the framework for investigating the molecular mechanisms and clinical benefit of these responses. This trial was registered at www.clinicaltrials.gov as #NCT05007860.

The full-text version of this article contains a data supplement.

Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia worldwide, accounting for approximately 114 000 new cases annually and 35 000 deaths.[1] Infectious complications are a leading cause of death, and the same mechanisms underlying enhanced infection risk contribute to impaired vaccine responses.[2-6] These include hypogammaglobulinemia, complement defects, and T-/natural killer cell dysfunction.[7,8] Vaccine responses are also affected by CLL therapy (eg, CD20 antibodies [CD20 Ab], Bruton's tyrosine kinase inhibitors [BTKi], and BH3 mimetics). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines are highly effective at reducing the risk of severe COVID-19,[9-11] but patients with CLL were excluded from these studies. CLL is associated with increased COVID-19 mortality,[12,13] and there is an urgent need to establish whether SARS-CoV-2 vaccines are effective in patients with CLL. Serologic responses were reported in 23.1% to 64.2% of patients with CLL following initial mRNA SARS-CoV-2 vaccination.[14-18] Whether SARS-CoV-2–specific antibodies are functional or accompanied by functional T-cell responses is unknown. Although a subset of vaccinated patients with CLL develop neutralizing antibodies against wild-type SARS-CoV-2,[18] antibodies also engage Fc receptors on immune effector cells, alone or in conjunction with neutralizing antibodies, to initiate an effective coordinated immune response against SARS-CoV-2. Herein, we prospectively evaluate the humoral and cellular immunogenicity of SARS-CoV-2 and pneumococcal conjugate (PCV13) vaccines in CLL and report the completed cohort of patients not on active CLL-directed therapy. We also measure functional spike-specific T-cell responses and perform antibody profiling to comprehensively analyze SARS-CoV-2 vaccine immunogenicity in CLL.

Methods

Patients

This prospective cohort study was approved by the Dana-Farber/Harvard Cancer Center Institutional Review Board and complied with Declaration of Helsinki and Good Clinical Practice Guidelines. All patients were enrolled at the Massachusetts General Hospital (MGH) Cancer Center and provided written informed consent.

Inclusion criteria were age ≥18 years and CLL/small lymphocytic lymphoma (World Health Organization criteria). We excluded HIV or primary immune deficiency disorder, planned initiation of CLL therapy within 2 months, prior CD20 Ab within 6 months, prior chemotherapy within 1 year, or any prior bendamustine or fludarabine. None had prior SARS-CoV-2 vaccination. We excluded patients with PCV13 ≤ 2 years or 2 to 5 years with protective titers for ≥50% of PCV13-specific *Streptococcus pneumoniae* IgG titers (supplemental Table 1). Prior COVID-19 infection was permitted and was assessed by history/serologies. Eligible patients were assigned to no active therapy (n = 30; completed primary analysis cohort) or active BTKi (n = 6; exploratory cohort) (supplemental Figure 1). All data collected for the study, including individual deidentified participant data and a data dictionary defining each field in the set, will be made available 6 months after publication.

Study assessments

Patient/disease characteristics, infection history, complete blood counts, immunoglobulins, and T-cell subsets were collected at baseline. Vaccine-specific serologies were collected before vaccination and 36 days after vaccination (median, 32 days; interquartile range, 27-35). *S pneumoniae* serologies were measured with the 23-serotype IgG assay. COVID-19 serologies were measured with a quantitative electrochemiluminescence immunoassay for detecting antibodies to SARS-CoV-2 spike protein receptor binding domain (RBD immunoassay; Roche Elecsys) and a qualitative electrochemiluminescence immunoassay for detecting antibodies to SARS-CoV-2 nucleocapsid antibody (N-immunoassay; Roche Elecsys). Due to logistical constraints in 2 cases, post–COVID-19 vaccine spike serologies were measured with the DiaSorin Liaison SARS-CoV-2 S1/S2 IgG assay (n = 1) or the Siemens SARS-CoV-2 total assay (n = 1).

Vaccine administration

SARS-CoV-2 vaccines were obtained from commercial supply and administered per the package insert, with product selection based on availability of BNT162b2 (Pfizer-BioNTech),[10] mRNA-1273 (Moderna),[11] or Ad26.COV2.S (Johnson & Johnson).[9] PCV13 (Wyeth) was obtained from commercial supply and administered per the package insert (supplemental Methods). SARS-CoV-2 and PCV13 vaccinations were not performed concurrently.

Patients without detectable spike protein antibody after vaccination (RBD immunoassay) were offered an additional dose of mRNA-1273 or BNT162b2 from investigational product surplus from a multicenter trial (#NCT04761822; supplemental Methods).

Antibody profiling

SARS-CoV-2 wild-type and variant-of-concern spike-specific antibody subclasses and isotypes from plasma samples were quantified using a bead-based multiplex immunoassay.[19] Bead-based flow cytometric assays were performed to measure antibody-dependent complement deposition (ADCD), antibody-dependent cellular phagocytosis (ADCP), and antibody-dependent neutrophil phagocytosis (ADNP) against SARS-CoV-2 wild-type and variant-of-concern spike in vaccinated patients with CLL and plasma samples in individuals with no history of cancer.[20-22] Individuals with no history of cancer (n = 14) provided informed consent under protocol MGH IRB 2020P003538, and plasma was collected after 2 doses of an mRNA SARS-CoV-2 vaccine.

Antigens and antigen biotinylation reaction. For ADCD, ADNP, and ADCP, SARS-CoV-2 wild-type (Lake Pharma) and variant spike antigen (B.1.1.7, B.1.351, B.1.617, P.1; Saphire Laboratory, La Jolla Institute for Immunology) were biotinylated with Sulfo-NHS-LC-LC biotin (Thermo Fisher Scientific), and excess biotin was removed by phosphate-buffered saline (PBS) buffer exchange in Zeba desalting 7-kDa cutoff spin columns (Thermo Fisher Scientific).

ADCD. ADCD was assessed as described previously.[21] Immune complexes were formed by incubating plasma samples diluted 1:10 in PBS and coupled biotinylated antigen with 1.0-μm red fluorescent neutravidin beads (Thermo Fisher Scientific) for 2 hours at 37°C in 96-well plates per antigen, in duplicate.

The beads were washed with PBS and incubated with reconstituted guinea pig complement (Cedarlane Labs) diluted in gelatin veronal buffer (Sigma-Aldrich) for 20 minutes at 37°C. The reaction was then stopped with 15 mM EDTA in PBS. Goat anti–guinea pig fluorescein isothiocyanate (FITC)-conjugated C3 polyclonal antibody (MP Biomedicals) diluted 1:100 in PBS was used to detect C3 deposition on the beads by flow cytometry (iQue 3; Sartorius). The median fluorescence intensity (MFI) of all bead-positive events in the FITC channel was analyzed with ForeCyt 8.1. Results were visualized with GraphPad Prism 9.

ADNP. ADNP assay using isolated primary human neutrophils was performed as described previously.[22] Biotinylated antigens were individually coupled to 1.0-μm yellow-green, fluorescent neutravidin-labeled microspheres (Thermo Fisher Scientific). Immune complexes were formed by incubating the biotinylated antigen-coupled beads with plasma samples diluted 1:50 in PBS for 2 hours at 37°C in 96-well plates per antigen tested, in duplicate. White blood cells were isolated from whole blood of healthy donors, collected by the Ragon Institute of MGH, Massachusetts Institute of Technology, and Harvard, coagulated with acid citrate dextrose. Two donors were used as experimental replicates. Red blood cells were lysed by mixing the blood at a 1:10 ratio with ammonium-chloride-potassium lysis buffer (Thermo Fisher Scientific). White blood cells and neutrophils were pelleted by centrifugation and washed with cold PBS. Cells were diluted to a final concentration of 250 000 cells/mL in R10 media (RPMI-1640; Sigma) supplemented with 10% Fetal bovine serum, 2 mM L-glutamine, and 100 U/mL of penicillin/streptomycin. Immune complexes were washed in PBS to remove unbound antibodies before adding 50 000 white blood cells in complete R10 per well and incubated for 1 hour at 37°C. The cells were stained with CD66b-V450/PacBlue AB (BioLegend) for 20 minutes, fixed with 4% paraformaldehyde, washed with PBS, and then resuspended in PBS. Neutrophil phagocytosis of beads was assessed by flow cytometry (iQue; Sartorius). The product of the percentage of neutrophils that phagocytosed beads and the fluorescent signal of phagocytosed beads (geometric MFI of bead-positive neutrophils) was calculated for each sample with ForeCyt 8.1 and reported as the phagocytotic score (phagoscore). Results were visualized with GraphPad Prism 9.

ADCP. Monocyte THP-1 cell-line-mediated phagocytosis assay was performed as described previously.[20] Immune complexes comprising of biotinylated antigen coupled to 1.0 μm yellow-green fluorescent, neutravidin-labeled microspheres (Thermo Fisher Scientific) and plasma antibodies diluted 1:100 in PBS were incubated for 2 hours at 37°C in 96-well plates per antigen, in duplicate. THP-1 monocytes (ATCC, TIB-202) were added at a concentration of 250 000 cells/well in R10 media (RPMI-1640; Sigma) supplemented with 10% fetal bovine serum, 2 mM of L-glutamine, 100 U/mL penicillin/streptomycin, 20 mM of HEPES, and 50 μM of β-mercaptoethanol. Cells were incubated with immune complexes for 16 hours at 37°C, 5% CO_2. Cells were then washed with PBS, fixed with 4% paraformaldehyde, and resuspended in PBS for flow cytometric acqusition (iQue; Sartorius). A phagoscore was calculated by dividing the product of percentage bead-positive cells and bead-positive MFI by 10^6 with ForeCyt 8.1. Results were visualized with GraphPad Prism 9.

Antibody subclass, isotype, and Fcγ-receptor titer. Magnetic microspheres (Luminex Corp.) were coupled to individual antigens per bead-region by a carbodiimide-NHS ester-coupling reaction (Thermo Fisher Scientific), as previously established.[19] The antigen-coupled beads were washed and incubated with plasma samples diluted in PBS (1:500 for IgG$_1$; 1:100 for IgG$_3$, IgA, and IgM; 1:1000 for Fc-receptor readouts) for 2 hours at 37°C in black, clear-bottom 384-well plates (Greiner Bio-One). The unbound antibodies were washed in assay buffer (0.05% Tween-20, 0.1% bovine serum albumin in PBS). Detection of antigen-bound antibodies of interest were detected with R-phycoerthrin (PE; Agilent Technologies) conjugated anti-human antibody for each subclass and isotype (IgG$_1$, IgG$_3$, IgA, and IgM; Southern Biotech). PE-streptavidin (Agilent Technologies) was conjugated to recombinant, biotinylated Fcγ-receptors (FcγR2A, FcγR2B, FcγR3A, and FcγR3B; Duke Protein Production Company). Each secondary detection was incubated with the immune complexes for 1 hour at room temperature. Plates were then washed in assay buffer and the beads were resuspended in QSol buffer (Sartorius) for flow cytometric acquisition (iQue; Sartorius) and analyzed with Fore-Cyt 8.1 software. MFI of PE is reported for antigen-specific antibody subclass or isotype, and Fcγ-receptor titers. Ebola virus glycoprotein, used as a negative control antigen, was purchased from IBT Bioservices.

Functional T-cell response assessment

Functional T-cell responses to SARS-CoV-2 vaccination were measured in peripheral blood collected before and after vaccination using interferon-γ (IFN-γ) responses to spike/nucleocapsid protein in an enzyme-linked immunospot (ELISpot) assay and a whole blood–based IFN-γ release assay (IGRA).[23]

Blood processing. Blood was collected (Lithium Heparin) and peripheral blood mononuclear cells (PBMCs) were isolated using ficoll density gradient centrifugation in a SepMate tube (Stem Cell Technologies).

Peptide pools/protein. Peptide pools spanning the entire spike and nucleocapsid sequence were obtained from JPT peptide solutions and consisted of 15mers with an 11 amino acid overlap, with guaranteed purity of >70%. The nucleocapsid pool (PM-WCPV-NCAP2) contained 102 peptides. The spike protein pool (PM-WCPV-S-3) was divided into pools A (158 peptides) and B (157 peptides, of which the last is a 17mer). Peptide pools were reconstituted in 50 uL of dimethyl sulfoxide (DMSO) and equal volume sterile PBS (Gibco). Working aliquots were stored at −20°C. S1-His protein (S1N-C52H3) and S2-His protein (S2N-C52H5) (Acro Biosystems) had >90% purity and were expressed in HEK293 cells. Proteins were reconstituted with sterile water to final concentration of 0.5 μg/μL and working aliquots were stored at −20°C.

ELISpot assay. Within 8 hours of venesection, PBMCs were resuspended in serum-free T-cell assay media (ImmunoSpot) and plated 2.5e5 cells/well of human IFN-γ ELISpot plate (ImmunoSpot). Cells were incubated with 1:1 DMSO:PBS (negative control); the spike A, spike B, or nucleocapsid peptide pools; or phytohemagglutinin (PHA; positive control). Antigen wells were set up in duplicate. PHA (Sigma-Aldrich) was added as a positive control at a final concentration of 5 μg/mL. Peptide pools contained each peptide at a final concentration of 2 μg/mL. Negative control wells contained equivalent volume of

Table 1. Baseline patient characteristics

		Cohort		
	Total, n = 36 (%)	No active CLL therapy, n = 30 (83)	Active BTKi, n = 6 (17)	P
Age at registration				
Median (range, y)	62 (31-86)	60 (31-86)	68 (58-76)	.23*
Age < 65 y	19 (53)	17 (57)	2 (33)	.39†
Age ≥ 65 y	17 (47)	13 (43)	4 (67)	
Sex				
Female	14 (39)	10 (33)	4 (67)	.18†
Male	22 (61)	20 (67)	2 (33)	
Infection history				
Pulmonary infection				
No	30 (83)	25 (83)	5 (83)	>.99†
Yes	6 (17)	5 (17)	1 (17)	
SARS-CoV-2 infection				
No	35 (97)	29 (97)	6 (100)	>.99†
Yes	1 (3)	1 (3)	-	
IVIG therapy				
No	36 (100)	30 (100)	6 (100)	>.99†
Yes	–	–	–	
Disease characteristics				
Rai stage				
0	29 (81)	25 (83)	4 (67)	.21†
1-2	6 (17)	5 (17)	1 (17)	
3-4	1 (3)	–	1 (17)	
Immunoglobulin heavy chain gene				
Unmutated	11 (31)	6 (20)	5 (83)	.01†
Mutated	13 (36)	13 (43)	–	
Missing	12 (33)	11 (37)	1 (17)	
CLL treatment status				
Treatment-naïve				
No	11 (31)	5 (17)	6 (100)	<.001†
Yes	25 (69)	25 (83)	–	
Number of prior lines				
Median (range)	0 (0-2)	0 (0-2)	1 (1-2)	<.001†
Laboratory parameters				
ALC				
Median (range)	13.9 (0.5-142.9)	14.5 (0.5-42.9)	4.6 (1.6-141.0)	.83*
ALC ≤ 24 000/µL	26 (72)	22 (73)	4 (67)	>.99†
ALC > 24 000/µL	10 (28)	8 (27)	2 (33)	
Immunoglobulin G				
Median (range)	761 (395-1858)	761 (395-1413)	734 (500-1858)	.85*
Immunoglobulin M				
Median (range)	42 (10-1157)	42 (10-1157)	32 (12-193)	.36*
Immunoglobulin A				
Median (range)	94 (19-495)	102 (19-495)	79 (34-398)	.77*
Absolute CD3+ T-cell count				
Median (range)	1797 (460-3947)	1729 (460-3947)	2735 (1784-3796)	.05*
Missing	5 (14)	4 (13)	1 (17)	

ALC, absolute lymphocyte count; IGHV, immunoglobulin heavy chain gene; IVIG, intravenous immunoglobulin; –, none.
*Wilcoxon rank-sum test.
†Fisher's exact test.

Table 1. (continued)

	Total, n = 36 (%)	Cohort		P
		No active CLL therapy, n = 30 (83)	Active BTKi, n = 6 (17)	
CD4+ T-cell count				
Median (range)	1268 (339-2743)	1127 (339-2743)	1656 (746-2185)	.12*
Missing	2 (6)	2 (7)	–	
Vaccination history				
COVID-19 vaccine type				
BNT162b2	14 (39)	13 (43)	1 (17)	.29†
mRNA-1273	13 (36)	11 (37)	2 (33)	
Ad26.COV2.S	9 (25)	6 (20)	3 (50)	

ALC, absolute lymphocyte count; IGHV, immunoglobulin heavy chain gene; IVIG, intravenous immunoglobulin; –, none.
*Wilcoxon rank-sum test.
†Fisher's exact test.

DMSO and PBS as peptide pools. Plates were incubated at 37°C for 16 to 20 hours and developed according to manufacturer's instructions before counting (ImmunoSpot CoreS6 ELISpot counter). Background spot forming unit (SFU) count was subtracted from the antigen wells spot count. Average SFU for spike pool A, spike pool B, and nucleocapsid pool is reported. In some instances, responses to spike pool A and B are added together to better reflect responses to the entire spike protein. In cases with no spot responses to antigen, the PHA well was always positive. The median background of the negative control was 0 SFU/2.5 × 10^5 cells (range 0-6). The positive threshold of average 6 SFU per 2.5 × 10^5 PBMC (after background subtraction) was previously described.[23]

IGRA. Within 8 hours of venesection, 1 mL of blood was aliquoted into sterile tubes with loose-fitting caps. For the negative condition, 20 μL of sterile water was added; for the positive control, 18 μL of PHA (1 μg/μL stock) and 2 μL of Cell Activation Cocktail (Biolegend) were added. Whole protein spike S1 subunit and spike S2 subunit of SARS-CoV-2 were thawed and immediately added (20 μL) to individual tubes for a final concentration of 5 mg/mL. Samples were carefully mixed before incubating at 37°C for 16 to 24 hours. Samples were then centrifuged and plasma isolated and stored at −20°C prior to IGRA testing (Qiagen). Manufacturer-specified acceptance criteria were always met. A positive threshold of ≥0.3 IU/mL IFN-γ (after subtraction of background in the negative control) to either S1 or S2 subunit was previously described.[23]

Statistical analyses

The primary end point was frequency of serologic response. Serologic response to SARS-CoV-2 vaccination was defined as ≥4-fold increase (ie, for patients with serologic evidence of prior infection) or seroconversion (negative [<0.40 U/mL] to ≥0.80 U/mL) with the RBD immunoassay. To control for prior SARS-CoV-2 infection, total nucleocapsid antibody was measured before and after vaccination using the *N*-immunoassay. Serologic response to PCV13 was defined as ≥2-fold increase or seroconversion (nonprotective to protective titer for ≥50% PCV13-specific serotypes). One patient was deemed ineligible for PCV13 analyses but included in COVID-19 analyses.

The sample size was calculated to provide adequate power for testing the primary end points (serologic response to SARS-CoV-2/PCV13 vaccines). A 1-sample binomial test was used to test the null hypothesis that ≤30% would achieve each primary end point, which was based on the previously reported serologic response rate (SRR) of pneumococcal vaccines in CLL.[24] SRRs associated with SARS-CoV-2 vaccines in patients with CLL were not established at study start. With 30 patients, there was 81.9% power to test 50% (alternative) vs 30% (null) using a 1-sided significance level of 0.084. This design required that ≥13 patients achieve the primary end point to be declared promising.

Patient/disease characteristics were summarized using descriptive statistics (median/range for continuous variables; numbers/percentages for categorical variables). Immune responses were tabulated/summarized descriptively. Frequencies/proportions were calculated with 95% exact binomial confidence intervals.

The following covariates were considered in the univariate analyses for SRR after SARS-CoV-2 vaccination: age, vaccine type, absolute lymphocyte count (ALC), CD3 T cells, CD4 T cells, IgG, immunoglobulin heavy chain gene status, Rai stage, and pulmonary infection ≤3 years. We used established cutoffs for age (≥65 years),[25,26] Rai stage (0 vs ≥1), and IgG (<600 mg/dL). We used univariate recursive partitioning to identify a robust cutoff for association of ALC (>24 000/μL; 10 000 models were fit with bootstrap resampling, with the median chosen as the cut point). CD3/CD4 T-cell cutoffs were based on median values. Associations of response with covariates were conducted using univariable logistic regression and reported as odds ratios with 95% confidence intervals.

Results

Patient characteristics

From August 2020 to August 2021, 36 patients with CLL/small lymphocytic leukemia were enrolled (supplemental Figure 1). The median age was 62 years (range, 31-86), 61% (22/36) were men, and 97% (35/36) had Rai stage of 0 to 1 (Table 1). The primary cohort was comprised of 30 patients not on active therapy (25 treatment-naïve and 5 previously treated). Most received mRNA-based SARS-CoV-2 vaccines (24/30; 80%), and 20% (6/30) received the adenovirus-based SARS-CoV-2 vaccine. A secondary BTKi cohort was comprised of 6 patients on active BTKi (Table 1).
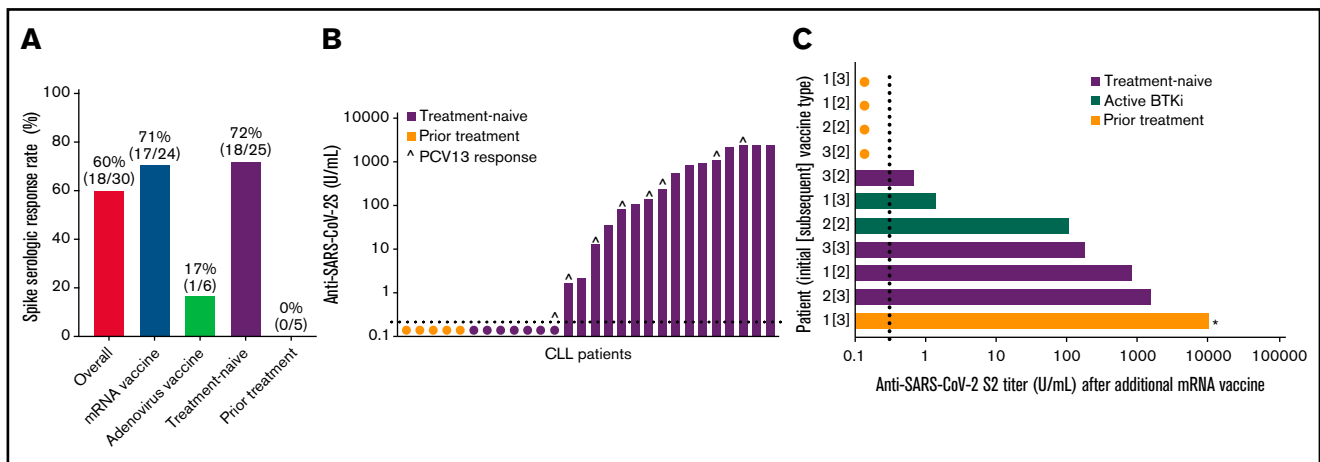
Figure 1. Humoral responses to SARS-CoV-2 vaccination in patients with CLL. (A) Anti–SARS-CoV-2 spike antibody response rate (RBD immunoassay) in patients with CLL not on active therapy (n = 30) by vaccine type and treatment history. One patient developed COVID-19 after enrollment but prior to vaccination and had a >4-fold spike antibody titer increase with vaccination and thus was counted as a response. (B) Distribution of anti–SARS-CoV-2 spike antibody responses in patients not on active therapy. Each circle or bar represents an individual patient and is color coded based on disease history. Dotted line represents RBD immunoassay cutoff for a detectable result (≥0.40 U/mL), with undetectable results (<0.40 U/mL) indicated as circles and results within the measuring interval (≥0.40 to >2500 U/mL) indicated as bars. Caret (^) indicates a serologic response to PCV13. Two patients with positive qualitative spike antibody results are not included here. (C) Distribution of anti–SARS-CoV-2 spike antibody responses (n = 11) following an additional mRNA vaccine dose. Each bar or circle represents an individual patient and is color coded based on disease history. Initial and subsequent SARS-CoV-2 vaccine types (1, Ad26.COV2.S; 2, mRNA-1273; 3, BNT162b2) are indicated on the y-axis, with the subsequent vaccine type placed in brackets after the initial vaccine type. Dotted line represents RBD immunoassay cutoff for a detectable result (≥0.40 U/mL), with undetectable results (<0.40 U/mL) indicated as circles and results within the measuring interval (≥0.40 to >2500 U/mL) indicated as bars. Every patient received 1 additional dose of mRNA vaccine except for patient indicated by an asterisk (*), who received 2 doses. (A-C) Prior treatment refers to CD20 antibody therapy plus venetoclax $^{+/-}$ BTKi within the past 2 years.

Serologic response to SARS-CoV-2 vaccination

The overall anti–SARS-CoV-2 SRR after SARS-CoV-2 vaccination in patients with CLL not on therapy was 60% (18/30; Figure 1A; supplemental Table 2; supplemental Figure 2) and was higher in treatment-naïve (72% [18/25]) than previously treated patients (0% [0/5]; Figure 1A). Among treatment-naïve patients who received an mRNA vaccine, the SRR was 77% compared with 33% for adenovirus vector-based vaccine. Of patients with CLL not on active therapy who responded to SARS-CoV-2 vaccination, the median spike antibody level was 402.00 U/mL (range, 1.72 to >2500 U/mL [upper limit of assay]; Figure 1B). Of 6 patients on a BTKi, the SRR was 33% (2/6; supplemental Table 3; supplemental Figure 3A-C).

Efficacy of a SARS-CoV-2 mRNA additional vaccine dose in seronegative patients

Patients who remained seronegative after SARS-CoV-2 vaccination (2 doses of mRNA vaccine [n = 6] or 1 dose of Ad26.COV2.S [n = 5]) were offered an additional dose of mRNA vaccine (BNT162b2 or mRNA-1273). Of 11 patients who received an additional SARS-CoV-2 vaccine dose, the SRR was 55% (6/11), and an additional patient achieved a detectable RBD immunoassay but was considered a nonresponder given titer <0.80 U/mL (Figure 1C). Of 6 patients with seroconversion after an additional mRNA vaccine dose, 3 (50%) previously received Ad26.COV2.S and 3 (50%) previously received an mRNA vaccine. Overall, of 28 patients not on therapy who received ≥1 dose of an mRNA vaccine (2 mRNA doses [n = 19], 3 mRNA doses [n = 5], or 1 Ad26.COV2.S vaccine plus 1 mRNA dose [n = 4]), the SRR was 79% (22/28).

Predictors of serologic response to SARS-CoV-2 vaccination in patients with CLL

In a preplanned univariate analysis to identify baseline covariates associated with serologic response to SARS-CoV-2 vaccination, we found that ALC ≤24 000/µL ($P = .028$), absolute CD3 T-cell count greater than the median ($P = .021$), and use of an mRNA-based vaccine ($P = .035$) were associated with serologic response to SARS-CoV-2 vaccination in patients not on therapy (Figure 2). Treatment-naïve patients with ALC ≤24 000/µL demonstrated a higher SRR than patients with prior/current CLL treatment or ALC >24 000/µL in the absence of prior treatment (94% [15/16] vs 14% [1/7]; $P < .001$; Figure 3).

SARS-CoV-2 antibody and effector function profiling following mRNA vaccination

We performed detailed characterization of SARS-CoV-2 spike-directed antibodies and their functional activity after mRNA-based SARS-CoV-2 vaccination in 21 patients with CLL (18 not on active therapy, 3 on BTKi; Figure 4). Using a bead-based multiplex immunoassay, we detected SARS-CoV-2 spike IgG₁, IgG₃, IgM, and IgA antibodies in all tested patients, in most cases at titers and binding capacities similar to vaccinated non-CLL controls, with the exception of lower IgG₃ titers and higher FcγR3B binding in patients with CLL (Figure 4A-B; supplemental Figure 4). Spike-specific ADCP responses to wild-type SARS-CoV-2 virus were preserved in patients with CLL compared with non-CLL controls, whereas ADCD and ADNP responses were slightly diminished (Figure 4D-E). IgG, IgM, and IgA subclasses, Fcγ-receptor binding, and spike-specific ADCP, ADNP, and ADCD responses were demonstrated against the α (B.1.1.7), β (B.1.351), γ (P.1), and δ (B.1.617)
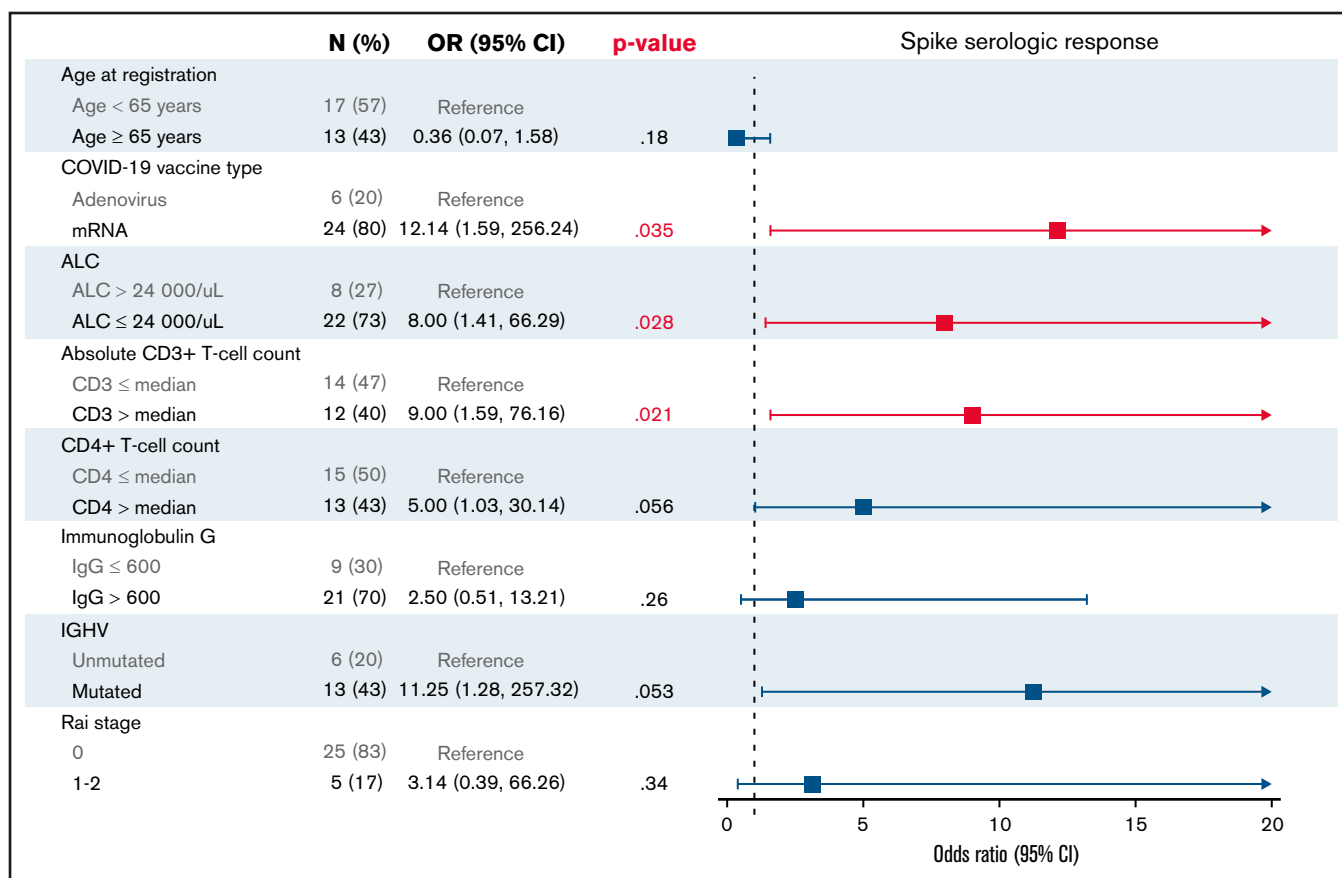
Figure 2. Clinical characteristics associated with SARS-CoV-2 vaccine response. Forest plot of univariable logistic regressions of characteristics associated with a spike serologic response (RBD immunoassay) to SARS-CoV-2 vaccination in patients not on any CLL therapy (n = 30). The odds ratio reflects the presence of the variable compared with the absence.

variants (supplemental Figure 4). As expected, SARS-CoV-2 spike IgG$_1$ levels appeared higher (1.17-log; P = NS), and ADCP (1.06-log; P = NS), ADNP (1.13-log; P = NS), and ADCD (1.35-log; P = .0126) responses appeared more robust among patients with CLL with RBD immunoassay response (Figure 4C).

Functional T-cell responses after SARS-CoV-2 vaccination

Among 20 patients with CLL (16 not on active therapy, 4 on BTKi) with samples for T-cell assays, 80% (16/20) had functional T-cell responses to SARS-CoV-2 spike protein, including 12/16 by ELI-Spot and IGRA and 4/16 by IGRA alone (Figure 5A-B; supplemental Figures 3C and 5). Of 9 patients who did not achieve a serologic response on RBD immunoassay, 7 (78%) demonstrated a functional T-cell response (Figure 5A). Notably, 2 patients without an RBD immunoassay or functional T-cell response had an ALC >24 000/μL (Figure 5A).

Breakthrough SARS-CoV-2 infections

With a median follow-up of 263 days (interquartile range, 241-280) from first SARS-CoV-2 vaccine, 3 patients developed SARS-CoV-2 infection, and all patients are alive (supplemental Table 4). Two patients (1 previously treated and 1 active BTKi) required hospitalization and received dexamethasone (n = 2), remdesivir (n = 2) ± casirivimab/imdevimab (n = 1). Both hospitalized patients were

initially vaccinated with Ad26.COV2.S with undetectable postvaccine titers (<0.04 U/mL) and received an additional dose of mRNA vaccine on study (subsequent titers of <0.04 and 1.39 U/mL). A third patient had mild upper respiratory symptoms and convalesced at home. This patient had received 3 doses of BNT162b2 with undetectable titers before and after the third dose.

Serologic response to PCV13 vaccination

PCV13 appeared less immunogenic than SARS-CoV-2 vaccines in the same cohort of patients, with a PCV13 SRR of 29% (8/28) in patients not on active therapy, including 35% (8/23) among treatment-naïve patients and 0% (0/5) among previously treated patients, and 20% (1/5) in patients on active BTKi therapy (Figure 6; supplemental Figure 3A). Of 9 patients with a PCV13 response, 8 (89%) had a SARS-CoV-2 vaccine response (Figure 1B; supplemental Figure 3B).

Discussion

This prospective study demonstrated that 60% of patients with CLL not on active therapy exhibited a serologic response to SARS-CoV-2 vaccination using a clinically validated RBD immunoassay, and responses appeared more frequently in patients who were treatment-naïve (72%) or received an mRNA vaccine (71%). Among treatment-naïve patients, an ALC ≤24 000/uL was strongly
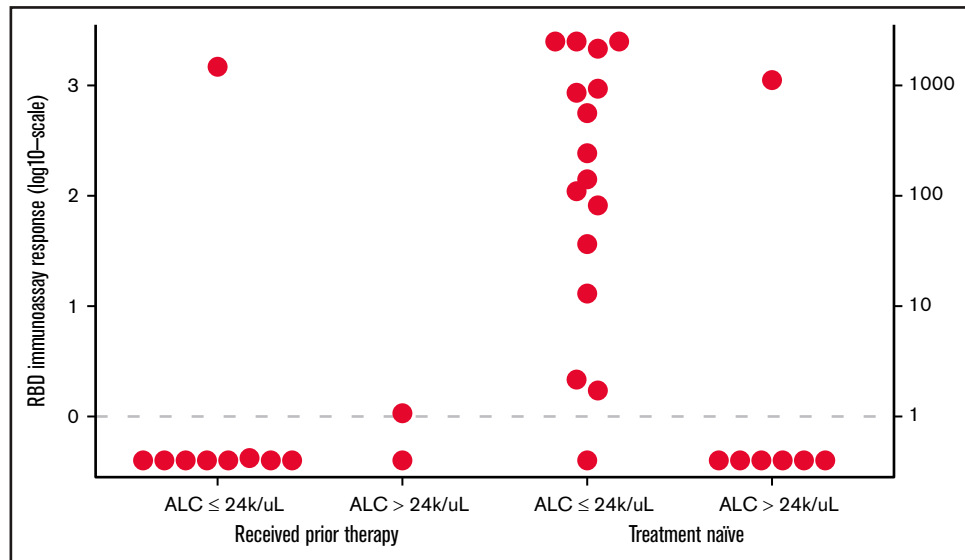
Figure 3. SARS-CoV-2 vaccine response by absolute lymphocyte count and treatment history. Spike antibody serologic response value by RBD immunoassay (y-axis) plotted against absolute lymphocyte count and treatment history (x-axis). Each circle corresponds to an individual patient (n = 34). P = .001 by Kruskal–Wallis rank-sum test comparing the distributions of the continuous data among the 4 groups; P < .001 for Wilcoxon rank-sum test comparing 2 groups (treatment-naïve patients with ALC ≤ 24 000/μL vs ALC > 24 000/μL and/or current or prior therapy). Patients enrolled in both cohorts were included in this analysis. Two patients without quantitative spike antibody serologies were not included in the plot.

associated with serologic response (94% vs 14%; P < .001). Importantly, we found that most (80%) tested patients had functional SARS-CoV-2 specific T-cell responses, including most patients who lacked detectable SARS-CoV-2 antibodies after vaccination. Additionally, a higher sensitivity bead-based multiplex immunoassay identified SARS-CoV-2 antibodies in all tested patients, and we confirmed Fc-receptor binding and spike-specific antibody effector functions against wild-type and variant SARS-CoV-2 viruses (α, β, γ, and δ).

This study met its primary end point (serologic response to SARS-CoV-2 vaccination), with SRRs of 60% overall, including 72% among treatment-naïve patients, consistent with previous reports in patients with CLL without recent or ongoing therapy (55% to 94%).[15,16,18] PCV13 did not meet its co-primary end point with an SRR of 28%, which is consistent with previous reports,[4,5] supporting that this is a representative cohort of patients with CLL not on active therapy in terms of immune responses to vaccination. *S pneumoniae* is a major cause of morbidity and mortality among patients with CLL, and these data underlie the urgency to develop better vaccines against common pathogens that affect immunocompromised patients. Notably, whereas 88% (7/8) of off-treatment patients who responded to PCV13 demonstrated a SARS-CoV-2 vaccine response, only 39% (7/18) of patients who responded to SARS-CoV-2 vaccination demonstrated a PCV13 response. These data support the development of novel vaccine technologies against other pathogens (eg, *S pneumoniae*, *Haemophilus influenzae*, and influenza).

An ALC ≤ 24 000/μL was associated with a higher likelihood of serologic response to SARS-CoV-2 vaccination (73% vs 27%; P = .028). We combined both cohorts and observed that treatment-naïve patients with an ALC ≤ 24 000/μL had a higher SRR than those with prior/current therapy and/or high ALC (94%

vs 14%; P < .001). Others have also found that lymphocytosis is associated with fewer serologic responses to SARS-CoV-2 vaccines,[18] and these data are consistent with prior reports that advanced disease burden is an independent risk factor for infections among treatment-naïve patients with CLL.[27] External validation of this association between the ALC and SARS-CoV-2 vaccine efficacy is necessary, and further studies to identify the mechanisms underlying this finding are warranted.

Prior studies of SARS-CoV-2 vaccine efficacy in CLL have focused on immunoassay-based serologic responses but did not answer whether antibodies measured after vaccination are capable of effector functions, if certain antibody titers confer protective immunity, and whether spike antibodies are accompanied by spike-specific T-cell responses. To address these knowledge gaps, which have left us unable to effectively counsel CLL patients as to whether they are protected against SARS-CoV-2, we performed detailed analyses of humoral and T-cell immunity after SARS-CoV-2 vaccination. Using a bead-based multiplex immunoassay with higher sensitivity than traditional immunoassays,[28] we identified spike-specific antibody subsets after mRNA-based vaccination in all tested patients with CLL. Fc-receptor binding and spike-specific antibody effector functions (eg, cellular and neutrophil phagocytosis and complement deposition) were observed in all tested patients with CLL, although they appeared more robust among patients with a positive RBD immunoassay result. We also demonstrated that 80% of tested patients with CLL developed functional spike-specific T-cell responses, including 78% of patients with CLL without detectable spike antibodies on an RBD immunoassay, a group enriched for prior B-cell–depleting therapies.

These data provide a model for comprehensively assessing vaccine immunogenicity in other high-risk populations excluded from pivotal trials. Taken together, our findings confirm that post-vaccination RBD immunoassay antibody testing underestimates the immunogenicity
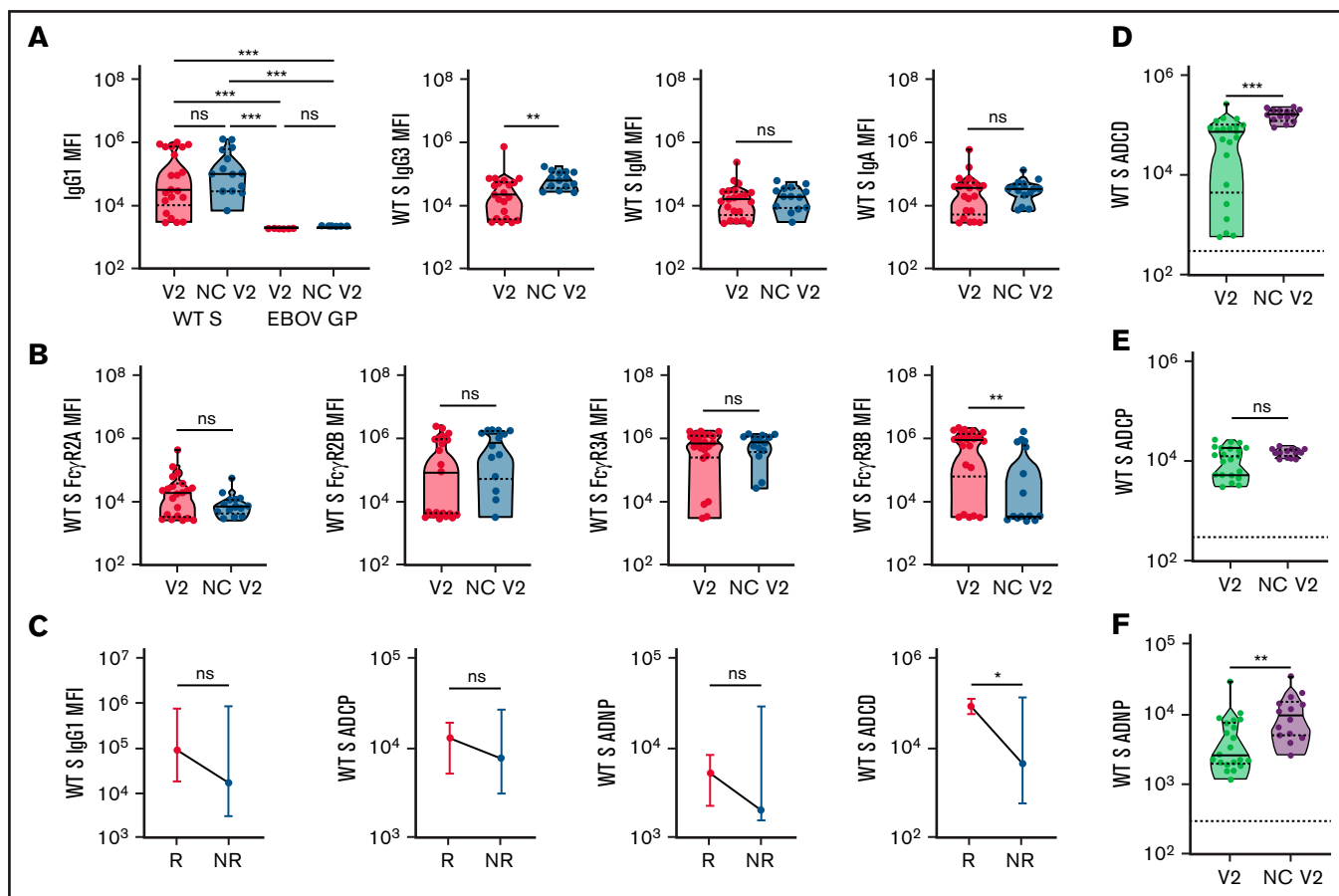
Figure 4. SARS-CoV-2 wild-type spike-specific antibody responses in patient with CLL or non-cancer (NC) donor plasma after 2 doses of SARS-CoV-2 mRNA vaccine. (A) SARS-CoV-2 spike-specific plasma IgG_1, IgG_3, IgM, or IgA titer from vaccinated patients with CLL (V2; n = 21) and NC donors (V2; n = 14) were analyzed with a bead-based multiplex immunoassay, represented as median florescence intensity (MFI) in log10. Antibody titers against Ebola virus glycoprotein (EBOV GP) were used as a negative antigen control readout. WT S, wild-type spike. (B) SARS-CoV-2 spike-specific plasma FcγR2A, FcγR2B, FcγR3A, or FcγR3B titers from vaccinated patients with CLL (V2; n = 21) and NC donors (V2; n = 14) were assessed with a bead-based multiplex immunoassay and analyzed as median florescence intensity (MFI) in log10. (C) Univariate analysis of RBD immunoassay responder (R; n = 12) and nonresponder (NR; n = 3) antibody features among vaccinated patients with treatment-naïve CLL. Circle represents the median of each group. SARS-CoV-2 spike-specific IgG_1 titer is reported as MFI in log10. Flow cytometric assays to determine SARS-CoV-2 spike-specific antibody-dependent THP-1 monocyte phagocytosis (ADCP), ADNP, or ADCD effector functionality were performed to measure the relative effector function. Cellular or neutrophilic phagocytosis is reported as phagoscore in log10. Complement deposition is reported as the MFI of C3-FITC–positive cells in log10. (D) Comparison of SARS-CoV-2 spike-specific ADCP functionality between vaccinated patients with CLL (V2; n = 21) and NC donors (V2; n = 14). Complement deposition is reported as the MFI of C3-FITC–positive cells in log10. (E) Comparison of vaccinated patient with CLL (V2; n = 21) or NC donor (V2; n = 14) antibody-dependent THP-1 monocyte-mediated phagocytosis functionality against SARS-CoV-2 spike. ADCP is reported as the phagoscore in log10. (F) Comparison of ADNP effector functionality against SARS-CoV-2 spike protein between vaccinated patients with CLL (V2; n = 21) and NC donors (V2; n = 14). ADNP is reported as the phagoscore in log10. (A-F) Postvaccine samples were available for 21 patients with CLL vaccinated with mRNA SARS-CoV-2 vaccines. Statistical significance of comparisons between the 2 groups was calculated using the nonparametric two-tailed Mann-Whitney *U* test with *P* value cutoffs indicated as follows: *P* = .1234 (ns); *P = .0332; **P = .0021; ***P = .0001. (A-B, D-F) The black solid line represents the median with dashed lines for the 95% confidence interval in the truncated violin plots. (D-F) The dotted line on the y-axis represents background PBS signal.

of SARS-CoV-2 vaccination and provides evidence of functional humoral and/or cellular immune responses after vaccination against SARS-CoV-2 in most patients with CLL. The significance of spike antibodies detectable only with higher sensitivity methods and/or presence of spike-specific T-cell responses without a positive RBD immunoassay warrant prospective validation. However, these data suggest that patients with CLL generate functional immune responses to vaccines, and novel vaccine strategies (eg, additional mRNA vaccine doses) should be studied to enhance protection against SARS-CoV-2 virus.

Of 11 patients with a negative RBD immunoassay after SARS-CoV-2 vaccination, 6 (55%) responded to an additional dose of mRNA-based vaccine. These data call into question RBD immunoassay–directed vaccine strategies but suggest that a 3-dose mRNA vaccine series or an mRNA vaccine after Ad26.COV2.S may be more immunogenic in patients with CLL. In a randomized trial of mRNA-1273 vs placebo in solid organ transplant recipients after primary vaccination, a third dose resulted in more serologic and T-cell responses.[29] Although our cohort given additional vaccine doses is limited by its small
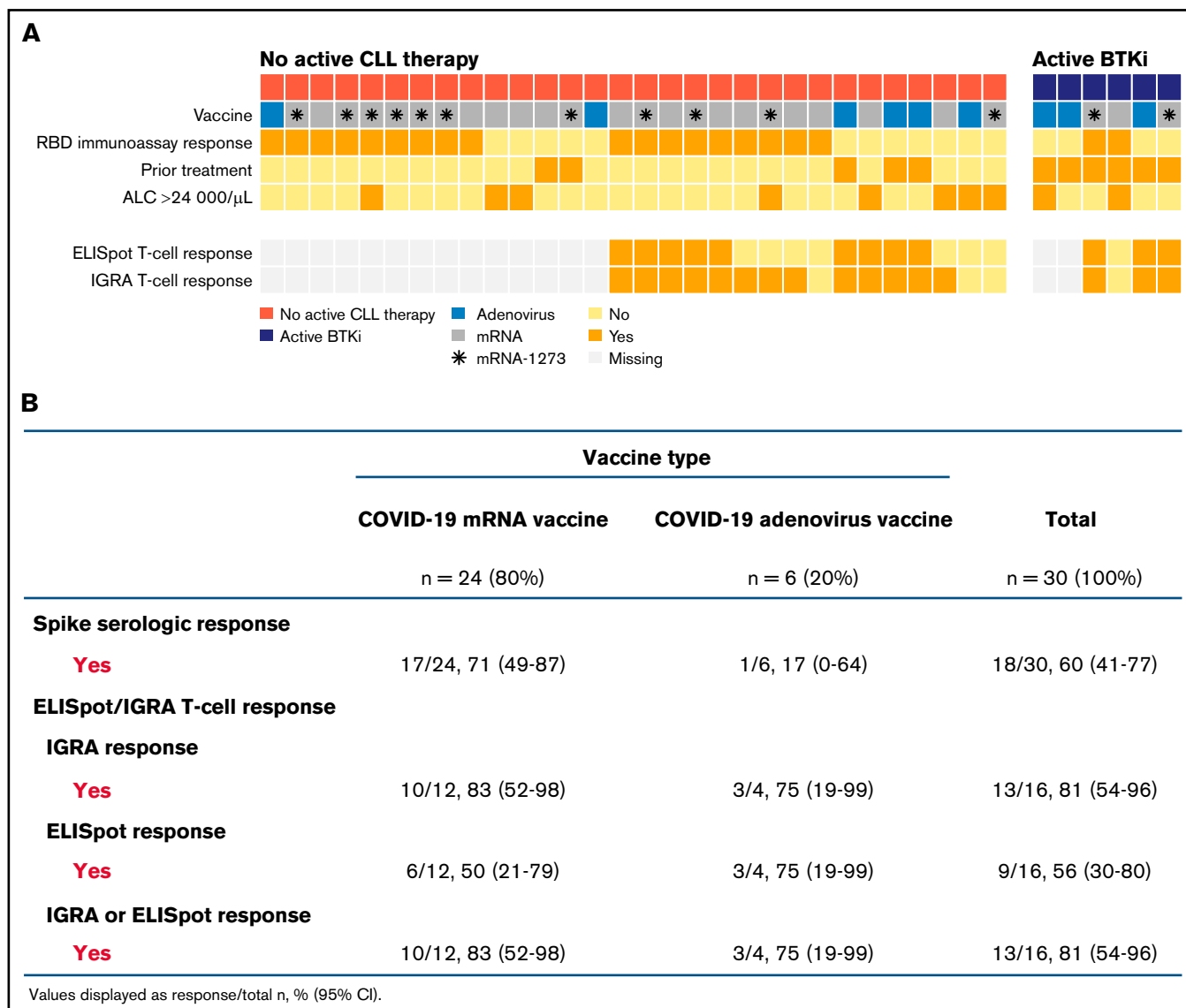
Figure 5. T-cell responses to SARS-CoV-2 vaccination in patients with CLL. (A) Co-occurrence plot of spike RBD immunoassay serologic responses with T-cell responses (ELISpot and IGRA), vaccine history, and clinical characteristics (n = 36). Shaded squares represent the presence of the indicated characteristic. Each column represents an individual patient. (B) Summary table of spike RBD immunoassay serologic and T-cell responses based on SARS-CoV-2 vaccine type (mRNA vs adenovirus) for patients not on any active therapy. (A-B) Postvaccine samples were available for 20 patients with CLL vaccinated with either adenovirus or mRNA SARS-CoV-2 vaccines.

sample size and lack of a control, our serologic conversion rate compares favorably with organ transplant recipients given an additional vaccine dose (33% to 44%)[30,31] and in seronegative patients with CLL given a third dose of BNT162b2 (23.8%)[32] and further supports the current Centers for Disease Control and Prevention recommendations to offer additional mRNA-based vaccine doses to immunocompromised patients, such as patients with CLL. Additional assessment, including serial antibody profiling and T-cell assays, should be obtained over time and with additional SARS-CoV-2 vaccine doses and may provide additional insight into the optimal SARS-CoV-2 vaccine type/frequency to ensure that patients with CLL achieve and maintain protective immunity to SARS-CoV-2 variants of concern.

Our study was not powered to detect differences between vaccines or treatment history to analyze risk of breakthrough infection; thus, randomized data and/or large well-controlled observational datasets are needed to determine the optimal SARS-CoV-2 vaccine strategy in patients with CLL. Because the Centers for Disease Control and Prevention recently updated their guidance to recommend that mRNA-based vaccines be preferred over Ad26.COV2.S in all adults, we recommend mRNA-based vaccines for patients with CLL. Because the functional analyses were conducted before the B.1.1.529 variant (o), they should be repeated against o spike antigen once feasible. An additional limitation of these data is that most of the enrolled patients were treatment naïve and Rai stage 0 or 1 and had preserved IgG levels, and whether these data are generalizable to patients with high Rai stage or hypogammaglobulinemia[18]
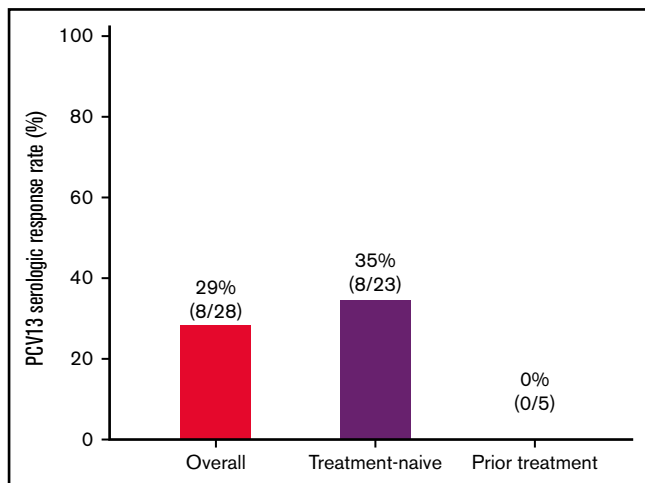
Figure 6. Humoral response to PCV13 vaccination in patients with CLL. PCV13 SRR in patients with CLL not on active therapy (n = 30) by treatment history.

warrants further evaluation. Although it is possible that the SRR would be significantly lower in a cohort of patients with a higher ALC, those with low ALC were enriched for prior therapy and/or active BTKi.

Strengths of our study include a prospectively powered design in a defined cohort of patients not on active therapy, comprehensive evaluation of SARS-CoV-2 vaccine immunogenicity, including functional T-cell responses, antibody Fc-receptor binding, and effector functions, paired PCV13/SARS-CoV-2 analyses, and RBD immunoassay response assessment after an additional mRNA vaccine dose in patients with a negative RBD immunoassay. To our knowledge, this is the most comprehensive analysis of SARS-CoV-2 vaccine immunogenicity in CLL to date. Whether these surrogates of SARS-CoV-2 vaccine immunogenicity (eg, RBD immunoassay, bead-based spike immunoassay, presence of antibody effector functions, and/or functional T-cells) predict protective immunity is unknown and warrants further study. Although we and others have reported that people with CLL generally have a lower SRR compared with people without cancer, we can detect antibodies capable of FcR binding and effector function in all patients receiving mRNA vaccines and functional spike specific T-cells in most patients, suggesting that novel vaccine strategies, including additional vaccine doses, may increase protection against SARS-CoV-2 infection. Our results may also lead to further studies of the mechanisms underlying functional T-cell and humoral responses after SARS-CoV-2 vaccination and whether they predict protective immunity in patients with CLL.

Acknowledgments

The authors are grateful to the patients who participated in this study. The authors also wish to acknowledge P.A.L., T.L.H., and J.M.C. for vaccine administration.

Authorship

Contribution: J.E.H., J.D.S., and J.S.A. designed the study, enrolled patients, analyzed data, and wrote the manuscript; J.S.M., S.F., and G.A. generated and analyzed the antibody profiling data; K.M.E.G., K.K., and M.V.M. generated and analyzed the functional T-cell data; R.A.R. performed statistical analyses; D.P., J.R., S.S., M.D., and C.R. coordinated the clinical trial; J.A.B., E.P.H., P.C.J., R.W.T., K.J.S., B.M.M., M.M.S., J.M.L., and J.I.W. enrolled and managed patients on trial; K.G.B., A.B., and A.D.J. oversaw additional vaccine doses and provided allergy input; C.N.K. provided infectious disease input; M.L. coordinated trial procedures and provided pharmacy input; and A.S.D. coordinated serologic testing.

Conflict-of-interest disclosure: E.P.H. reported consulting fees from Leuko and Intervention Insights. J.I.W. reported consulting fees from AbbVie. M.L. reported consulting fees from AbbVie, AstraZeneca, Epizyme, Fresenius Kabi, and Intervention Insights. C.N.K. reported data safety monitoring board membership for BeiGene and serves on the Centers for Disease Control and Prevention advisory committee on immunization practice. M.V.M. reported consulting fees from Adaptimmune, Agenus, Allogene, Arcellx, Astellas, AstraZeneca, Atara, Bayer, BMS, Cabaletta Bio, Cellectis, CRISPR Therapeutics, In8bio, Inc., Innovakine Therapeutics, Intellia Therapeutics, GlaxoSmithKline, Kite Pharma, Micromedicine/BendBio, Neximmune, Novartis, TCR2, Tmunity, Torque, and WindMIL, has equity or stock holdings in TCR2, Century Therapeutics, Ichnos, and Neximmune, received research support from CRISPR Therapeutics, Kite Pharma, Servier, and Novartis, serves on the board of directors for Ichnos Sciences and Bluebird Bio, and is an inventor on patents related to adoptive cell therapies, held by Massachusetts General Hospital and University of Pennsylvania, including some licensed to Novartis. G.A. is founder of SeromYx Systems, Inc. J.S.A. reported consulting fees from AbbVie, Bayer, Celgene, Gilead Sciences, Juno Therapeutics, Kite Pharma, Genentech, Amgen, Novartis, Karyopharm Therapeutics, Verastem Oncology, Janssen, Merck, and Seattle Genetics. J.D.S. reported consulting fees for AbbVie, Adaptive, AstraZeneca, Beigene, Bristol Myers Squibb, Seattle Genetics, TG Therapeutics, and Verastem, and research funding for investigator-initiated research from Adaptive Biotechnologies, Beigene, BostonGene, Genentech/Roche, GlaxoSmithKline, Moderna, and TG Therapeutics. The remaining authors declare no competing financial interests.

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References

1. Fitzmaurice C, Abate D, Abbasi N, et al; Global Burden of Disease Cancer Collaboration. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2017: a systematic analysis for the Global Burden of Disease Study. *JAMA Oncol.* 2019;5(12):1749-1768.

2. Sinisalo M, Aittoniemi J, Oivanen P, Käyhty H, Olander RM, Vilpo J. Response to vaccination against different types of antigens in patients with chronic lymphocytic leukaemia. *Br J Haematol.* 2001;114(1):107-110.

3. Pleyer C, Ali MA, Cohen JI, et al. Effect of Bruton tyrosine kinase inhibitor on efficacy of adjuvanted recombinant hepatitis B and zoster vaccines. *Blood.* 2021;137(2):185-189.

4. Pasiarski M, Rolinski J, Grywalska E, et al. Antibody and plasmablast response to 13-valent pneumococcal conjugate vaccine in chronic lymphocytic leukemia patients: preliminary report. *PLoS One.* 2014;9(12):e114966.

5. Mauro FR, Giannarelli D, Galluzzo CM, et al. Response to the conjugate pneumococcal vaccine (PCV13) in patients with chronic lymphocytic leukemia (CLL). *Leukemia.* 2021;35(3):737-746.

6. Hartkamp A, Mulder AH, Rijkers GT, van Velzen-Blad H, Biesma DH. Antibody responses to pneumococcal and haemophilus vaccinations in patients with B-cell chronic lymphocytic leukaemia. *Vaccine.* 2001;19(13-14):1671-1677.

7. Forconi F, Moss P. Perturbation of the normal immune system in patients with CLL. *Blood.* 2015;126(5):573-581.

8. Ravandi F, O'Brien S. Immune defects in patients with chronic lymphocytic leukemia. *Cancer Immunol Immunother.* 2006;55(2):197-209.

9. Sadoff J, Gray G, Vandebosch A, et al; ENSEMBLE Study Group. Safety and efficacy of single-dose Ad26.COV2.S vaccine against COVID-19. *N Engl J Med.* 2021;384(23):2187-2201.

10. Polack FP, Thomas SJ, Kitchin N, et al; C4591001 Clinical Trial Group. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. *N Engl J Med.* 2020;383(27):2603-2615.

11. Baden LR, El Sahly HM, Essink B, et al; COVE Study Group. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med.* 2021; 384(5):403-416.

12. Scarfò L, Chatzikonstantinou T, Rigolin GM, et al. COVID-19 severity and mortality in patients with chronic lymphocytic leukemia: a joint study by ERIC, the European Research Initiative on CLL, and CLL Campus. *Leukemia.* 2020;34(9):2354-2363.

13. Mato AR, Roeker LE, Lamanna N, et al. Outcomes of COVID-19 in patients with CLL: a multicenter international experience. *Blood.* 2020;136(10): 1134-1143.

14. Agha M, Blake M, Chilleo C, Wells A, Haidar G. Suboptimal response to COVID-19 mRNA vaccines in hematologic malignancies patients [published online ahead of print 7 April 2021]. *medRxiv.*

15. 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(23):3165-3173.

16. Roeker LE, Knorr DA, Thompson MC, et al. COVID-19 vaccine efficacy in patients with chronic lymphocytic leukemia. *Leukemia.* 2021;35(9): 2703-2705.

17. Greenberger LM, Saltzman LA, Senefeld JW, Johnson PW, DeGennaro LJ, Nichols GL. Antibody response to SARS-CoV-2 vaccines in patients with hematologic malignancies. *Cancer Cell.* 2021;39(8):1031-1033.

18. Benjamini O, Rokach L, Itchaki G, et al. Safety and efficacy of BNT162b mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia [published online ahead of print 29 July 2021]. *Haematologica.*

19. Brown EP, Dowell KG, Boesch AW, et al. Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles. *J Immunol Methods.* 2017;443:33-44.

20. Ackerman ME, Moldt B, Wyatt RT, et al. A robust, high-throughput assay to determine the phagocytic activity of clinical antibody samples. *J Immunol Methods.* 2011;366(1-2):8-19.

21. Fischinger S, Fallon JK, Michell AR, et al. A high-throughput, bead-based, antigen-specific assay to assess the ability of antibodies to induce complement activation. *J Immunol Methods.* 2019;473:112630.

22. Karsten CB, Mehta N, Shin SA, et al. A versatile high-throughput assay to characterize antibody-mediated neutrophil phagocytosis. *J Immunol Methods.* 2019;471:46-56.

23. Gallagher KME, Leick MB, Larson RC, et al. SARS -CoV-2 T-cell immunity to variants of concern following vaccination [published online ahead of print 3 May 2021]. *bioRxiv.*

24. Svensson T, Kättström M, Hammarlund Y, et al. Pneumococcal conjugate vaccine triggers a better immune response than pneumococcal polysaccharide vaccine in patients with chronic lymphocytic leukemia A randomized study by the Swedish CLL group. *Vaccine.* 2018;36(25): 3701-3707.

25. Christie A, Henley SJ, Mattocks L, et al. Decreases in COVID-19 cases, emergency department visits, hospital admissions, and deaths among older adults following the introduction of COVID-19 vaccine: United States, September 6, 2020-May 1, 2021. *MMWR Morb Mortal Wkly Rep.* 2021; 70(23):858-864.

26. International CLL-IPI working group. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. *Lancet Oncol.* 2016;17(6):779-790.

27. Williams AM, Baran AM, Meacham PJ, et al. Analysis of the risk of infection in patients with chronic lymphocytic leukemia in the era of novel therapies. *Leuk Lymphoma.* 2018;59(3):625-632.

28. Bennett M, Yoder S, Brady E, et al. A high-throughput liquid bead array assay confirms strong correlation between SARS-CoV-2 antibody level and COVID-19 severity. *iScience.* 2021;24(2):102052.

29. Hall VG, Ferreira VH, Ku T, et al. Randomized trial of a third dose of mRNA-1273 vaccine in transplant recipients. *N Engl J Med.* 2021;385(13): 1244-1246.

30. 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(7):661-662.

31. Werbel WA, Boyarsky BJ, Ou MT, et al. Safety and immunogenicity of a third dose of SARS-CoV-2 vaccine in solid organ transplant recipients: a case series. *Ann Intern Med.* 2021;174(9):1330-1332.

32. Herishanu Y, Rahav G, Levi S, et al. Efficacy of a Ttird BNT162b2 mRNA COVID-19 vaccine dose in patients with CLL who failed standard two-dose vaccination [published online ahead of print 3 December 2021]. *Blood.*