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TO THE EDITOR:

Cellular and humoral immune response to mRNA COVID-19 vaccination in subjects with chronic lymphocytic leukemia

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is of special concern to patients with chronic lymphocytic leukemia (CLL).[1,2] Over time, individuals with CLL experience impaired B-cell function and antibody production, leaving them at an increased risk for severe infection or death. Patients with CLL suffer immune dysregulation from the disease, which is further disrupted by the effects of CLL-specific treatments. There are 3 vaccines for SARS-CoV-2 approved in the United States,[3] with high immunogenicity in immunocompetent subjects.[4-6]

The postimmunization dynamics in patients with CLL are different from those observed in healthy subjects. Attenuated humoral responses to vaccination have been documented.[7-10] Patients with CLL have among the lowest immune responses, which are influenced by disease status, immunoglobulin levels, and active or recent therapies.[11-14] In particular, treatment with anti-CD20 monoclonal antibodies (mAbs) or Bruton tyrosine kinase inhibitors (BTKi's) is associated with poor vaccine response.[10,15]

In this longitudinal cohort study, we interrogated the cellular and humoral immune response to novel vaccine antigen BNT162b2 (Pfizer-BioNTech) or messenger RNA (mRNA)-1273 (Moderna), as well as the humoral recall response to measles, in 16 subjects with CLL. In response to vaccination, immunocompetent individuals generate an antigen-specific response that results in cellular and humoral memory that persists long after vaccination,[16] including $CD4^+$ T cells, $CD8^+$ T cells, and 2 distinct long-lived populations of B cells: long-lived plasma cells (LLPCs), and memory B cells (MBCs). LLPCs traffic to the bone marrow and continuously secrete the antibodies that make up polyclonal immune serum, whereas MBCs, which do not secrete antibodies, circulate in peripheral blood surveying for invading pathogens. MBCs are especially important in the face of waning antibody titers or the emergence of new variants that might escape neutralization by serum antibodies.[17]

We enrolled subjects who were $\geq$18 years of age and without a known history of COVID-19 infection, prior to receiving the Moderna or Pfizer-BioNTech 2-dose SARS-CoV-2 mRNA vaccine series. This study reports the presence and magnitude of humoral and cellular immune responses, including quantitative receptor-binding domain (RBD)-specific antibody titers, RBD-specific MBC frequency following in vitro stimulation, and functional tumor necrosis factor-α and interferon-γ–secreting spike (S) peptide-specific $CD4^+$ and CD8 T cells at baseline (prior to vaccination) and $\sim$1-month (24-103 days) following the 2-dose mRNA vaccination series.

We observed a 25% seroconversion rate. Four patients with vaccine-mediated antibody responses were diverse: 1 was treatment naive, 1 was receiving treatment with bcl-2 inhibitor, and 2 were under observation. Of the patients under observation, 1 was in remission, whereas the other had relapsed disease. When stratified by treatment, 50% of subjects currently under observation following treatment seroconverted compared with 12.5% of subjects currently receiving active treatment (Figure 1A; Table 1). Of the responders (4/16), 1 had never received anti-CD20 mAb treatment, and 3 had received treatment >12 months earlier, consistent with previous studies.[18] In an attempt to identify potential predictors of response, we evaluated a number of clinical factors, as well as immune profiling. Although no significant differences

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Requests for data sharing may be submitted to Stephen E. Spurgeon (spurgeos@ohsu.edu).

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

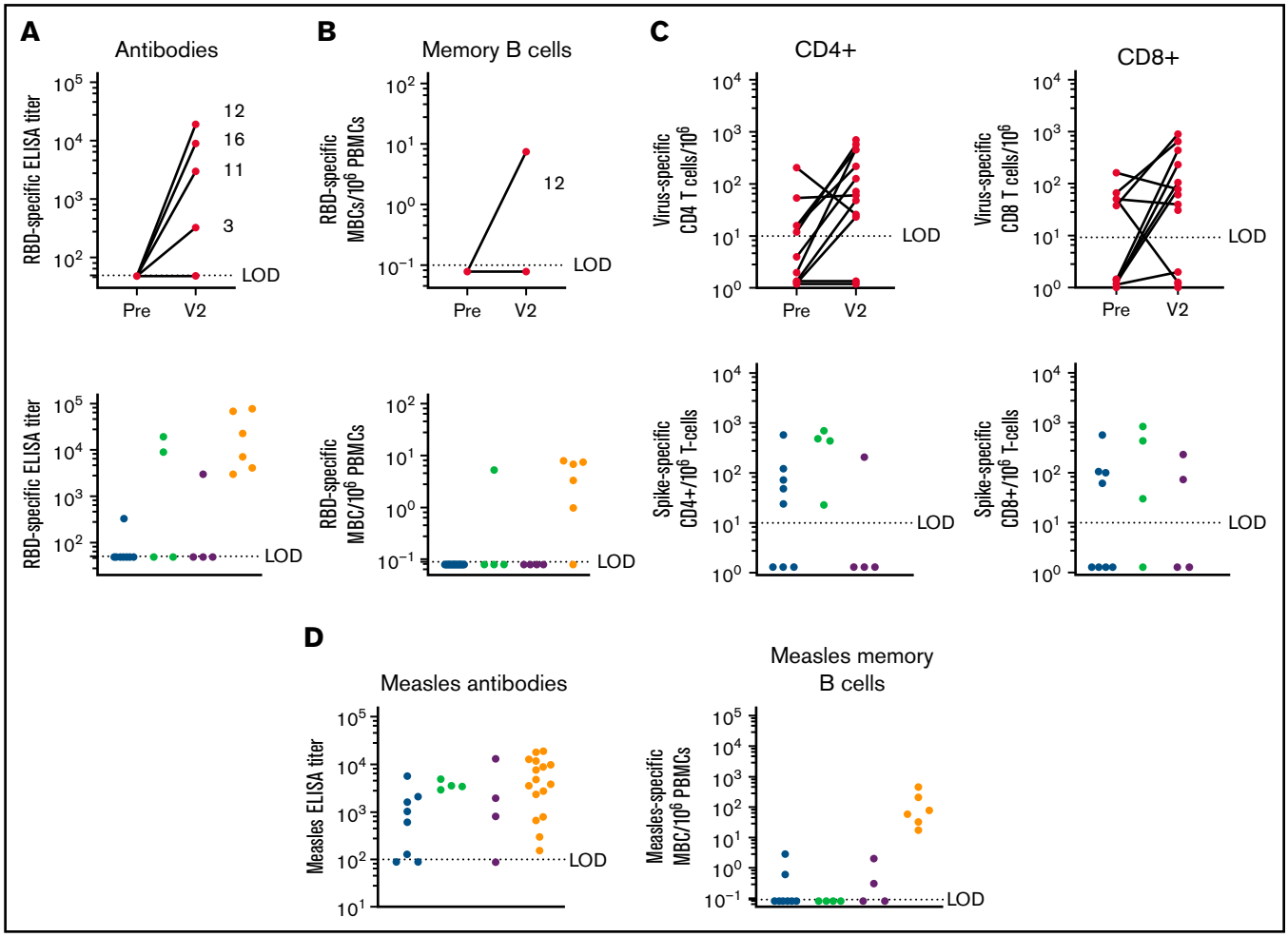

Figure 1. Immune response to vaccination. (A) Antibodies: RBD-specific end point enzyme-linked immunosorbent assay (ELISA) titer following COVID-19 mRNA vaccination: prior to vaccination and V2 following 2-dose vaccination series (24-103 days) (upper panel) is shown. Individual subject numbers are shown (3, 11, 12, and 16) for responders. RBD-specific ELISA titer stratified by treatment group; geometric mean titer (GMT) of responders is shown above the graph (lower panel). The limit of detection (LOD) is set at 50; samples below the LOD were given an arbitrary value of 49. Healthy subject samples were taken (13-28 days) following the 2-dose vaccination series (lower left panel). (B) RBD-specific memory B-cell frequency per 10^6 peripheral blood mononuclear cells (PBMCs) following COVID-19 mRNA vaccination: prior to vaccination and V2 (24-103 days) following the 2-dose vaccination series (upper panel). Only subject 12 developed an MBC response. RBD-specific MBC frequency stratified by treatment group (lower panel). Geometric mean titer of responders is shown above the graph. Healthy subject samples (247-264) post 2-dose vaccine series are included (lower right panel). LOD = 0.1; an arbitrary number (0.08) was assigned to samples below the LOD. (C) S-specific CD4 (left upper panel) and CD8 (right upper panel) T-cell frequency per 10^6 T cells following COVID-19 mRNA vaccination: prior to vaccination and V2 following 2-dose vaccination series (24-103 days) (lowerpanel). S-specific $CD4^+$ and $CD8^+$ response to vaccination: the increase in T-cell expansion from baseline, stratified by treatment group (lower panel). Geometric mean titer of responders is shown above the graph. LOD = 10; for subjects without a vaccine-specific response, an arbitrary value between 1.1 and 1.5 was assigned. (D) Humoral immune recall response to a childhood antigen (measles) in subjects with CLL and age/sex-matched healthy controls. Antibodies: measles-specific end point ELISA titer stratified by treatment group (left panel). LOD = 100; samples below the LOD were assigned an arbitrary value of 80. Geometric mean titer of responders is shown above the graph for each group. Memory B cells: measles-specific MBC frequency stratified by treatment group (right panel). Geometric mean frequency of responders is shown above the graph. LOD = 0.1; an arbitrary number between .05 and .1 was assigned to those samples. Red, active treatment; blue, observation after treatment; green, treatment naive; yellow, healthy age/sex-matched controls.

were appreciated, responders had overall higher immunoglobulin G (IgG) serum levels and lower absolute lymphocyte count (ALC), mean B-cell percentage, class-switched MBCs, and B1 B cells compared with nonresponders (supplemental Tables 3 and 4). Interestingly, only 1 subject (subject 12), who was in disease remission, with bcl-2 inhibitor treatment occurring >6 months prior to vaccination in combination with an anti-CD20 mAb treatment given >12 months prior to vaccination, exhibited an RBD-specific memory B-cell response. The observation that 3 of 4 patients with an RBD-specific antibody response did not have detectable RBD-specific MBCs is notable (Figure 1B). All subjects who had an RBD-specific antibody response also had an S-specific $CD4^+$ T-cell response, indicating that a population of T helper cells was available for B-cell priming.

SARS-CoV-2 S-reactive T cells were present at baseline in some of the subjects (Figure 1C). Subjects 5 and 8 and subjects 3 and 13 did not exhibit any expansion of S-responsive $CD4^+$ T cells and or and S-reactive $CD8^+$ T cells, respectively, following vaccination. This is consistent with previous reports of S-reactive T cells in naive

Table 1. Summary of subject immune response to mRNA COVID-19 vaccination and recall response to measles antigen

			Response to mRNA COVID-19 vaccine				Response to measles									
Subject ID	Age, y/sex	Vaccine	Ab	CD4	CD8	MBC	Ab	MBC	Treatment	CD20 Ab	ALC 1-4.8*	IgG 700-1600 mg/dL*	$CD19^+$ 4-17%*	IgD^-CD27^+ 5-21%*	CD4 30-60%*	CD8 10-30%*
1	62/Male	P	–	–	–	–	–	–	N	No	48.00	85	96.00	3.60	2.00	0.70
2	63/Female	P	–	–	+	–	+	+	N	No	21.00	NA	76.00	59.00	9.30	10.00
3	48/Male	?	+	+	–	–	–	–	C	Yes (≤12)	1.00	NA	2.10	17.00	39.00	33.00
4	77/Female	M	–	-	–	–	+	–	N	No	87.00	573	NA	NA	NA	NA
5	81/Female	P	–	-	+	–	+	+	C	Yes (>12)	5.80	NA	NA	NA	19.00	3.80
6	67/Male	M	–	+	+	–	+	+	C	No	2.90	918	30.00	2.30	41.00	19.00
7	60/Female	P	–	+	+	–	+	–	O (6)	Yes (>12)	1.60	NA	0.00	0.00	86.00	8.00
8	66/Male	P	–	-	–	–	+	–	C	Yes (>12)	0.43	526	3.00	22.00	35.00	47.00
9	65/Female	?	–	+	+	–	+	–	C	Yes (≤12)	1.30	NA	0.07	0.00	62.00	21.00
10	62/Female	P	–	+	-	–	–	–	C	Yes (>12)	30.00	262	65.00	2.40	23.00	8.00
11	63/Male	P	+	+	+	–	+	+	N	No	17.00	780	83.00	0.21	13.00	2.10
12	61/Male	P	+	+	+	–	+	–	O (6-12)	Yes (>12)	0.75	593	8.00	3.80	42.00	15.00
13	70/Male	P	–	+	–	–	+	–	C	Yes (>12)	5.80	101	45.00	1.50	20.00	20.00
14	65/Male	P	–	+	–	–	+	–	O (>12)	Yes (>12)	0.33	405	NA	NA	77.00	9.30
15	64/Male	M	–	–	+	–	+	–	C	Yes (>12)	1.70	100	0.41	0.94	26.00	42.00
16	75/Male	P	+	+	+	–	+	–	O (>12)	Yes (>12)	0.29	547	0.10	0.00	22.00	19.00

For T-cell–specific responses, "+" indicates an increase in S-specific T cells compared with baseline and "–" indicates no change (or a decrease) in S-specific T cells following vaccination. Current treatment status, CD20 Ab treatment, and clinical values were recorded at baseline (time of enrollment) when available.

Ab, antibody; ALC, absolute lymphocyte count; C, currently on treatment; M, Moderna; N, treatment naive; NA, baseline values were not available; O (6), observation, last treatment within 6 months; O (6-12), observation, 6 to 12 months since last treatment; O (>12), observation, >12 months since last treatment; P, Pfizer-BioNTech; ?, unknown; +, response above the LOD; –, response below the LOD.

*Normal range.

individuals without prior antigen exposure.[19] The cellular immune response seemed to be fairly robust compared with the humoral immune response in these subjects with CLL, consistent with previous studies.[20,21] We observed a 62.5% $CD4^+$ T-cell response and a 56% $CD8^+$ T-cell response. Four subjects had an S-specific $CD4^+$ response alone, 3 subjects had an S-specific $CD8^+$ response alone, and 6 subjects had $CD4^+$ and $CD8^+$ responses. Four of the 10 $CD4^+$ responders seroconverted, providing supporting evidence for the importance of $CD4^+$ T-cell help in generating a B-cell response.

Active treatment with BTKi's has a significant impact on B-cell survival, differentiation, and the development of an antigen-specific antibody response to novel antigen exposure. B cells are dependent on Bruton tyrosine kinase signaling for differentiation and proliferation signals, and immune response to novel antigens, either by natural infection or vaccination, is severely limited in these subjects[15]; however, recall to previously encountered antigens remains largely intact (Figure 1D). Seventy-one percent of subjects on BTKi's had a cellular immune response with $CD4^+$ and/or $CD8^+$ T cells. Whether this finding translates to an effective T-cell response associated with a clinical benefit is of interest. Because BTKi's are administered daily, further studies that evaluate the timing of vaccines or interruption of ongoing BTKi therapy in an attempt to enhance vaccine response are warranted. This approach showed success in patients with rheumatologic disease on immunosuppressive therapies.[22] Bcl-2 is a protein regulator of apoptosis, and preclinical data suggest that bcl-2 inhibition affects T-cell function.[23] The impact of ongoing bcl-2 inhibition with venetoclax remains an unanswered question that is worthy of additional study.

A recent study[15] reported an impaired vaccine response to novel antigens in patients with CLL, resulting in seroconversion in 28.1% of treatment-naive subjects and only 3.8% of patients on BTKi's. Compared with the humoral response to previously vaccinated antigens, the response was 41.5% in subjects on BTKi's and 59.1% for treatment-naive subjects, indicating that BTKi's disrupt the generation of novel immune responses but do not necessarily interfere with recall. We explored the recall response to measles and observed that 81% of subjects were seropositive for measles serum antibodies; subjects 3 and 10 were on active treatment with bcl-2 inhibitor and BTKi's, respectively, and 1 subject was treatment naive. This is a slightly higher response rate than was observed in a recent cross-sectional study of 959 patients[24] that detected a 63% measles seropositivity rate in subjects with hematological malignancies. The antibody response to measles seems to be largely unaffected in subjects with CLL, indicating that LLPCs responsible for maintaining circulating serum antibodies remain stable throughout CLL immune dysfunction and treatment. However, the MBC recall response to measles was highly disrupted in these subjects. Only 25% retained a detectable population of measles-specific MBCs: of these 4 subjects, 2 were on active treatment (subjects 5 and 6), and 2 were treatment naive. Although a population of measles-specific MBCs was detected in these subjects, the frequencies were lower than those observed in age/sex-matched healthy controls (geometric mean frequency, 78.4).

In summary, the results of this study provide a thorough evaluation of the humoral and cellular immune response to the initial 2-dose mRNA COVID-19 vaccine series in patients with CLL. Our results highlight the limitations of serology studies alone in defining vaccine-mediated immune responses, particularly in this immune-dysregulated patient population. Larger longitudinal studies incorporating clinical outcomes in vaccinated patients with CLL, as well as the impact of a third booster or heterologous vaccine, are needed.

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