REGULAR ARTICLE

blood advances

BTK inhibitors impair humoral and cellular responses to recombinant zoster vaccine in CLL

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Key Points

- Antibody and T-cell immune responses to RZV are impaired in patients with TN CLL and to a greater extent in patients treated with a BTKi.
- Although antibody and cellular immune response are often concordant, 39% of patients without antibody response mounted a T-cell response.

Vaccinations effectively prevent infections; however, patients with chronic lymphocytic leukemia (CLL) have reduced antibody responses following vaccinations. Combined humoral and cellular immune responses to novel adjuvanted vaccines are not well characterized in CLL. In an open-label, single-arm clinical trial, we measured the humoral and cellular immunogenicity of the recombinant zoster vaccine (RZV) in CLL patients who were treatment naïve (TN) or receiving Bruton tyrosine kinase inhibitor (BTKi) therapy. The primary endpoint was antibody response to RZV (≥fourfold increase in anti-glycoprotein E [anti-gE]). Cellular response of gE-specific $CD4^+$ T cells was assessed by flow cytometry for upregulation of ≥2 effector molecules. The antibody response rate was significantly higher in the TN cohort (76.8%; 95% confidence interval [CI], 65.7-87.8) compared with patients receiving a BTKi (40.0%; 95% CI, 26.4-53.6; $P = .0002$). The cellular response rate was also significantly higher in the TN cohort (70.0%; 95% CI, 57.3-82.7) compared with the BTKi group (41.3%; 95% CI, 27.1-55.5; $P = .0072$). A concordant positive humoral and cellular immune response was observed in 69.1% (95% CI, 56.9-81.3) of subjects with a humoral response, whereas 39.0% (95% CI, 24.1-54.0) of subjects without a humoral response attained a cellular immune response ($P = .0033$). Antibody titers and T-cell responses were not correlated with age, absolute B- and T-cell counts, or serum immunoglobulin levels (all $P > .05$). RZV induced both humoral and cellular immune responses in treated and untreated CLL patients, albeit with lower response rates in patients on BTKi therapy compared with TN patients. This trial was registered at www.clinicaltrials.gov as #NCT03702231.

Introduction

Immune dysregulation is a hallmark of chronic lymphocytic leukemia (CLL), making these patients vulnerable to infectious complications. Infections remain a significant cause of morbidity and mortality in patients with CLL.[1] Reactivation of latent varicella zoster virus (VZV) in the form of shingles, and postherpetic neuralgia is frequently associated with hematologic malignancies, including CLL.[2,3] The live zoster vaccine (Zostavax) is no longer available in the United States and has been superseded by the recombinant, adjuvanted VZV vaccine (RZV) because of its improved efficacy and safety in immunocompromised

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Requests for data sharing may be submitted to Clare Sun (clare.sun@nih.gov).

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

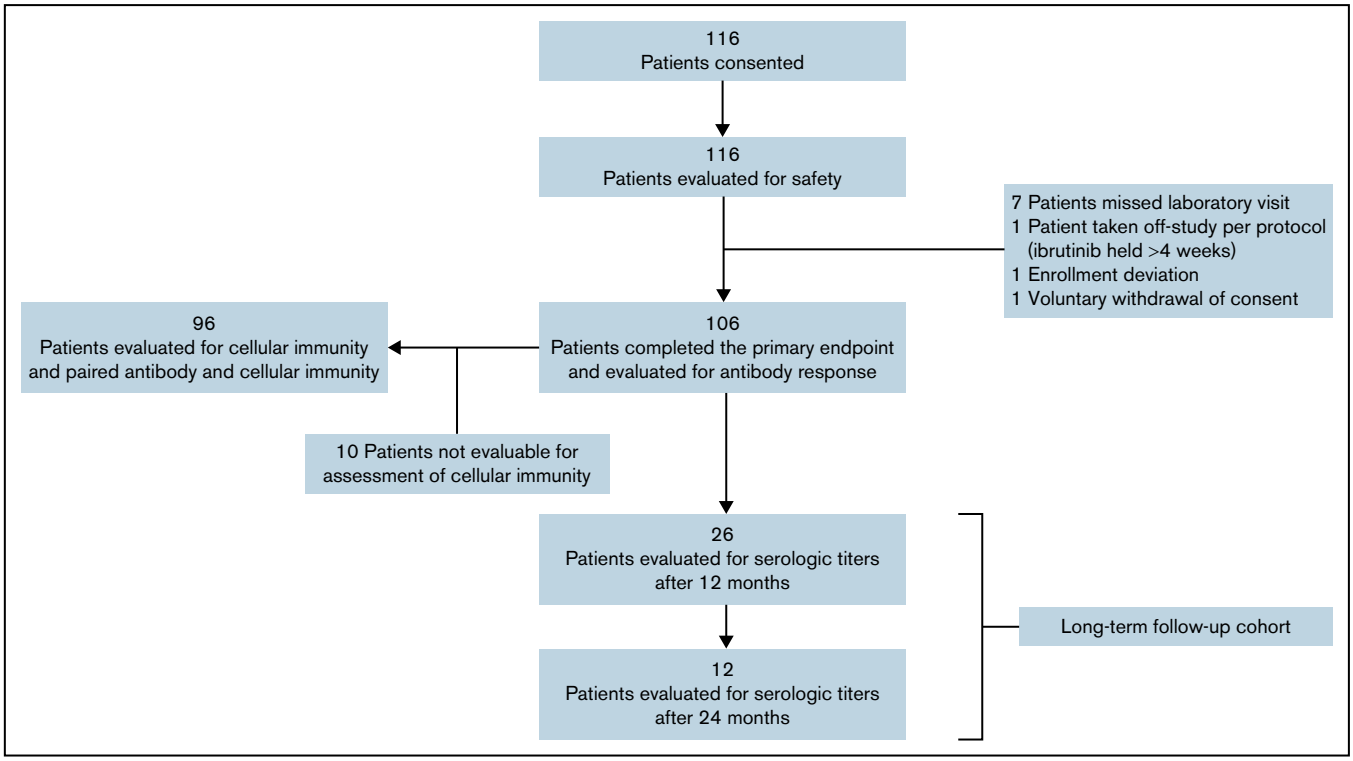

Figure 1. CONSORT diagram.

patients.[4,5] RZV induces vaccine responses in cancer patients,[6] but there is a paucity of data guiding the best use of RZV for patients with CLL. A large study investigating RZV in hematologic malignancies demonstrated that up to 80% of patients mounted an immune response to RZV[6]; notably, patients with CLL were excluded from the primary analysis.

We previously reported that a cohort of CLL patients who were treatment naïve or receiving treatment with a Bruton tyrosine kinase inhibitor (BTKi) have decreased humoral immune responses following RZV vaccination, with responses ranging between 41.5% and 59.1%.[7] Humoral responses are an essential part of immunity and are a common surrogate marker of protection in clinical vaccine studies. However, antigen-specific T-cell responses have recently received more attention. They appear crucial to host defense, may improve survival following coronavirus disease 2019 (COVID-19) viral infection, and are detectable after COVID-19 vaccination.[8,9] In the context of shingles, antiviral T cells are important in suppressing VZV reactivation.[10] Cellular immunity may play an outsized compensatory role in patients with hematologic malignancies because these diseases and their treatment frequently impair B cells and antibody production. The effect of vaccination on cellular immune responses in patients with CLL is not well characterized and has been reported variably between 41.0% and 87.5% in relatively small studies.[11,12] Therefore, gaining an enhanced understanding of both humoral and cellular immunity in patients with CLL who are treatment naïve or receiving targeted therapies can inform vaccination strategies in this high-risk patient population.

BTKis are a mainstay of treatment for CLL.[13,14] However, BTKis interfere with B-cell receptor signaling and may suppress antibody immune responses. For example, in patients with CLL receiving the BNT162b2 messenger RNA (Pfizer-BioNTech) COVID-19 vaccine, 32 of 58 (55%) treatment-naïve (TN) patients achieved a humoral response, compared with only 8 of 50 (16%) patients treated with a BTKi.[15] We previously reported interim results of vaccination studies in patients with CLL testing de novo immune response to the recombinant hepatitis-B vaccine (HEPLISAV-B) and recall response to RZV. Only 1 of 26 (3.8%) patients on continuous BTKi achieved a humoral response to HEPLISAV-B, compared with 9 of 32 (28.1%) patients with TN CLL. Consequently, the HEPLISAV-B study was stopped prematurely. In the interim analysis, responses to RZV were seen in both TN and BTKi-treated patients and study accrual continued to the predefined cohort size. Here, we present the results of the entire cohort vaccinated with RZV, report on both antibody and cellular immune responses, compare response rates in TN patients with those on continuous BTKi therapy, and present data on long-term persistence of antibody titers.

Methods

Study design and patient population

This prospective, open-label, investigator-initiated phase 2 trial investigated the efficacy and safety of the recombinant RZV vaccine (SHINGRIX, GlaxoSmithKline Biologicals) in patients with CLL that had either TN CLL or being treated with BTKis. Eligible patients had CLL or small lymphocytic lymphoma diagnosed per the international workshop on CLL guidelines.[16] Patients were previously untreated (active surveillance) or received BTKi monotherapy (ibrutinib or acalabrutinib) for at least 6 months before administration of the first vaccine dose. Key exclusion criteria were patients with Richter transformation, uncontrolled/symptomatic infection, intravenous or

Table 1. Baseline characteristics

	RZV (n = 106)		
	TN (n = 56)	BTKi (n = 50)	*P*
Age, median (IQR), y	66.0 (57.0-71.0)	66.0 (59.8-74.0)	.2682
Sex, n (%)			
Female	23 (41.1%)	19 (38.0%)	.7469
Male	33 (59.9%)	31 (62.0%)	
Disease/treatment status, n (%)			
Time from diagnosis, median (IQR), mo	56.5 (28-110)	102 (73-157)	.0008
Number of therapies before BTKi initiation			
0	–	30 (60.0%)	–
1	–	17 (34.0%)	–
2	–	3 (6.0%)	–
BTKi received			
Ibrutinib	–	26 (52.0%)	–
Acalabrutinib	–	24 (48.0%)	–
Duration on BTKi, median (IQR), mo	–	41.5 (18-65)	–
Best response to BTKi therapy			
Overall response rate	–	50 (100%)	–
Complete response	–	8 (16.0%)	–
Laboratory parameters, median (IQR)			
Absolute lymphocyte count, 1000 cells/μL	23.10 (9.40-60.27)	3.08 (1.76-7.47)	<.0001
β-2-microglobulin, mg/L	2.1 (1.6-2.6)	2.2 (1.8-2.8)	.5472
Immunoglobulin G, mg/dL	669 (551-838)	505 (401-819)	.0113
Immunoglobulin A, mg/dL	115 (45-173)	69 (40-115)	.0425
Immunoglobulin M, mg/dL	30 (16-56)	24 (13-47)	.1859
CD3 cell count, cells/μL	2807 (2023-4415)	1319 (1041-1727)	<.0001
CD4 cell count, cells/μL	1796 (1052-2620)	804 (617-1107)	<.0001
CD8 cell count, cells/μL	930 (517-1629)	449 (274-639)	<.0001

IQR, interquartile range.

subcutaneous immunoglobulin replacement within 3 months before vaccination, concomitant immunosuppressive therapy (eg, systemic steroids, radiotherapy, chemotherapy), and hereditary or AIDS unrelated to CLL. Patients must have had no active, symptomatic VZV or herpes zoster infection within 12 months before vaccination, no exposure to the live VZV vaccine (ZOSTAVAX) within 12 months, and no prior exposure to the RZV vaccine. The study was approved by the National Institutes of Health institutional review board. All patients provided written informed consent.

Evaluation and treatment

RZV was given at baseline and at 3 months via intramuscular injection. Humoral and cellular vaccine response was assessed 6 months after the first vaccine administration (window period: ±15 days). Following each vaccine dose, patients received an adverse event (AE) diary (supplement 1). Subjects documented any local (injection site) or systemic adverse effects that started within 7 days after receiving the first and second vaccine dose. Subjects noted adverse effects on the following scale: mild (grade 1), moderate (grade 2), severe (grade 3). Additionally, subjects had the option to participate in long-term follow-up and receive annual assessment of antibody titer.

Outcomes

The primary endpoint of antibody response was the rate of seroconversion (≥fourfold rise in VZV anti-glycoprotein E [anti-gE] blood immunoglobulin G titer) measured via luciferase immunoprecipitation assay.[17] Cellular immune response was assessed by intracellular

Table 2. Antibody and cellular response based on treatment status

	TN			BTKi			
Response type	Evaluable, N	Yes, N (%) [95% CI]	No, N (%) [95% CI]	Evaluable, N	Yes, N (%) [95% CI]	No, N (%) [95% CI]	*P*
Antibody (n = 106)	56	43 (76.8) [65.7-87.8]	13 (23.2) [12.2-34.3]	50	20 (40.0) [26.4-53.6]	30 (60.0) [46.4-73.6]	.0002
Cellular (n = 96)	50	35 (70.0) [57.3-82.7]	15 (30.0) [17.3-42.7]	46	19 (41.3) [27.1-55.5]	27 (58.7) [44.5-72.9]	.0072
Both (n = 96)	50	29 (58.0) [44.3-71.7]	21 (42.0) [28.3-55.7]	46	9 (19.6) [8.1-31.0]	37 (80.4) [69.0-91.9]	.0002

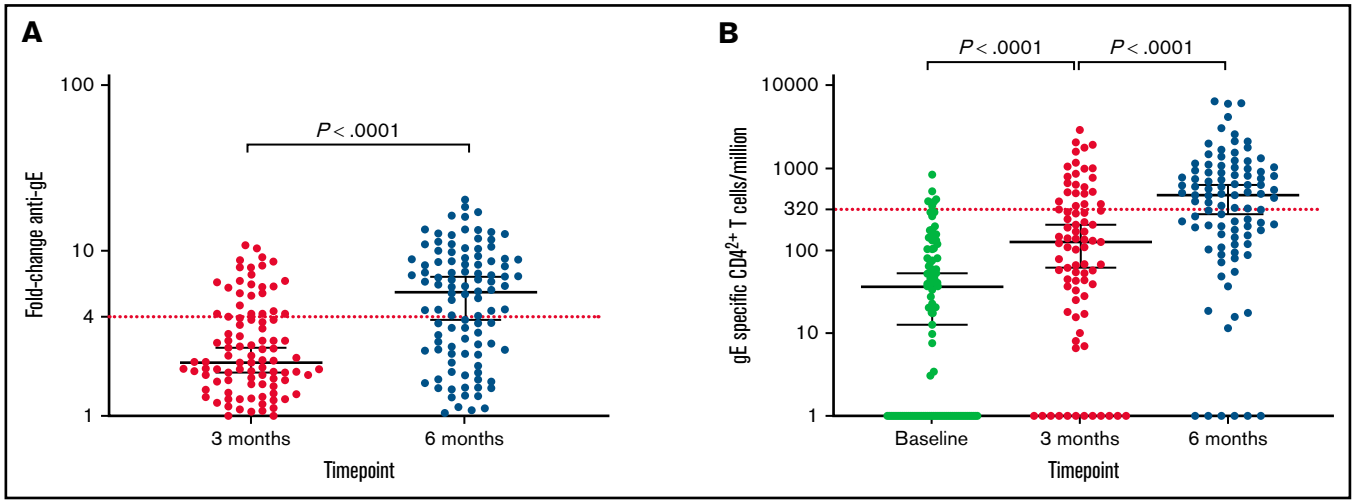

Figure 2. Antibody and cellular responses. (A) Fold-rise in VZV anti-gE IgG level compared with baseline at 3 and 6 months. (B) gE Specific CD4^{2+} cells per million CD4^{+} T cells at each timepoint. Bars indicate median and 95% CI. The dashed red lines represent the response threshold (≥fourfold rise in anti-gE titer) or >320 gE-specific CD4^{2+} T cells/million CD4^{+} T cells). At 6 months, 2 patients had a titer >320 gE-specific CD4^{2+} T cells/million CD4^{+} T cells, but did not achieve >twofold increase in titer required for a positive response.

cytokine staining and flow cytometric analysis of gE-specific CD4^{+} T cells expressing upregulation of ≥2 effector molecules (interferon-γ, interleukin-2, tumor necrosis factor-α, and/or CD40 ligand), termed CD4^{2+}. Cryopreserved peripheral blood mononuclear cells (PBMCs) were used for cellular response assessment. Based on pilot experiments demonstrating interference of CLL cells with T-cell assays, PBMCs with ≥10% CLL cells (n = 89) underwent CD19^{+} depletion. PBMCs (2×10^6 per well) were stimulated with a gE peptide pool (1 μg/mL each peptide; 0.3% final dimethyl sulfoxide concentration). The gE peptide pool consisted of overlapping peptides (134 × 15-mers; 11 amino acid overlap) spanning the extracellular domain and signal peptide region (amino acids 1-546) of VZV Dumas strain gE. The negative control consisted of 0.3% dimethyl sulfoxide. Additional control stimuli included ultraviolet-inactivated VZV and ultraviolet-treated HET lysate (mock)[18] and PHA-P. Cells were permealized and stained with fluorochrome-labeled monoclonal antibodies to human CD3, CD4, and CD8 as well as activation markers with anti-human CD40L, interferon-γ, interleukin-2, and tumor necrosis factor-α. Events were recorded with BD Fortessa and analyzed with FlowJo (v10 for Mac; BD). Cellular response was defined as ≥twofold rise over baseline and ≥320 net gE-specific CD4^{2+} cells per million CD4^{+} T cells.[19] Additional information on cellular response methodologies can be found in supplement 2. The safety endpoint was the safety and tolerability of the RZV vaccine among subjects with CLL.

Table 3. Positive cellular responses based on antibody responses (Yes/No) and treatment status. Denominator for percentage calculations is the % antibody response (Yes/No)

	Cellular response present		
Antibody response	**TN**	**BTKi**	**All patients**
Yes	29 of 38 (76.3%)	9 of 17 (52.9%)	38 of 55 (69.1%)
No	6 of 12 (50.0%)	10 of 29 (34.5%)	16 of 41 (39.0%)

Statistical analysis

A total of 50 subjects were required to estimate the rate of seroconversion in each of the TN and BTKi cohorts. This target enrollment of 100 subjects would ensure a power of 87% to detect a 30% difference in primary response rates between the 2 cohorts, hypothesizing a 40% or higher response rate in the TN cohort. Descriptive statistics were used to report vaccine response rates. Mann-Whitney test and Fisher exact test compared titers and response rates between different groups. Spearman ρ was used to measure the correlation between vaccine responses and clinical characteristics. Multivariate analysis was carried out based on logistic regression models. All analyses were conducted using GraphPad Prism, version 8.4.2.

Results

Patient characteristics and disposition

Between December 2018 and March 2020, 116 patients were enrolled and evaluated for safety (Figure 1). One enrollment deviation occurred, and 1 patient voluntarily withdrew consent from the study; both patients received 1 vaccine dose and were included in the safety analysis. Seven patients were not evaluable for the primary endpoint because of missed laboratory visits, predominantly from travel restrictions during the COVID-19 pandemic. One patient held ibrutinib for >4 weeks and was taken off the study, per protocol. Table 1 summarizes baseline characteristics. The median age in both groups was 66.0 years, with a male predominance typical for CLL. Fifty-six (52.8%) patients were TN, whereas 50 (47.3%) patients received a BTKi. TN patients had a shorter median time since diagnosis of CLL compared with patients receiving BTKi (56.5 months vs 102 months; $P = .0008$). All patients on BTKi were in remission. Among patients treated with a BTKi, 20 (40.0%) patients had received at least 1 prior line of therapy.

Table 4. Antibody and cellular response based on type of BTKi (ibrutinib or acalabrutinib)

	Ibrutinib			Acalabrutinib		
Response type	Evaluable, N	Yes, N (%)	No, N (%)	Evaluable, N	Yes, N (%)	No, N (%)
Antibody (n = 50)	26	11 (42.3)	15 (57.7)	24	9 (37.5)	15 (62.5)
Cellular (n = 46)	23	9 (39.1)	14 (60.9)	23	10 (43.5)	13 (56.5)
Both (n = 46)	23	3 (13.0)	20 (87.0)	23	6 (26.1)	17 (73.9)

Immunogenicity

A total of 106 patients were evaluable for primary response assessment of a ≥fourfold increase in VZV antibody titer (Table 2). The humoral response rate to RZV was significantly higher in the TN cohort (76.8%) compared with patients receiving a BTKi (40.0%; $P = .0002$). The prevaccination anti-gE geometric mean concentration was 27 113 luciferase light units (LLUs) (95% confidence interval [CI], 21 785-33 744) and increased to 63 458 LLUs (95% CI, 49 118-91 984) 3 months after the first vaccine and to 124 034 LLUs (95% CI, 93 784-164 040) at 6 months. There was no difference in baseline anti-gE titers between the TN and BTKi cohort (TN: median 35 548 LLUs [95% CI, 20 232-49 064] and BTKi median 19 610 LLUs [95% CI, 9826-37 356]; $P = .0677$). The median fold-change of anti-gE titers at 3 and 6 months was 2.09 (95% CI, 1.84-2.58) and 5.62 (95% CI, 3.81-6.94), respectively (Figure 2A). The median fold-change of anti-gE titers at 6 months was 7.76 (95% CI, 6.09-8.91) in the TN cohort and 3.31 (95% CI, 2.48-4.34) in the BTKi cohort ($P < .0001$).

Ninety-six patients were evaluable for assessment of cellular immunogenicity (Table 2). Similar to humoral responses, the rate of cellular immunity was significantly higher in the TN cohort (70.0%) compared with patients treated with a BTKi (41.3%; $P = .0072$). Median $CD4^{2+}$ T-cell frequencies before vaccination were 36 cells per million (95% CI, 13-53), 130 cells per million (95% CI, 62-207) after 1 vaccination, and 475 cells per million (95% CI, 277-623) after completing the vaccine series (Figure 2B). There was no difference in $CD4^{2+}$ T-cell frequencies at baseline between the TN and BTKi cohort (TN: median 38 cells/million [95% CI, 3-54]; BTKi: median 33 cells/million [95% CI, 1-73]; $P > .05$). Among all patients with paired antibody and cellular responses, 69.1% of subjects with a serologic response also had a positive cellular immune response, whereas 39.0% of subjects attained a cellular immune response in absence of a serologic response ($P = .0033$) (Table 3). Among subjects with a negative serologic response and a positive cellular immune response, 40.0% were TN (n = 6) and 60.0% (n = 9) received a BTKi.

There was no significant difference in humoral or cellular responses between ibrutinib or acalabrutinib treatment (Table 4). Furthermore, there was no significant difference in humoral or cellular responses in BTKi-treated patients that were receiving BTKis as first-line or subsequent line therapy. The fold-rise in antibody titers and the frequency of $CD4^{2+}$ T cells at 6 months were weakly positively correlated in the entire patient population ($\rho = 0.22$ [95% CI, 0.03-0.40]; $P = .0293$) (Figure 3). There was no statistically significant correlation between the fold-rise in antibody titers and the frequency of $CD4^{2+}$ T cells at 6 months in the TN and BTKi cohort, respectively ($P > .05$) (Figure 3). Antibody titers (fold-change) and $CD4^{2+}$ T-cell frequencies at 6 months were not correlated with age, serum β-2 microglobulin, absolute lymphocyte count, absolute peripheral blood $CD19^{+}$, $CD3^{+}$, $CD3^{+}/CD4^{+}$ T cell or $CD3^{+}/CD8^{+}$ T cells/μL counts, serum immunoglobulin levels (IgA, IgG, and IgM), or duration of BTKi exposure in treated patients (all $P > .05$). Similar to the results of Cunningham et al,[19] we did not observe any increase in $CD8^{+}$ T-cell responses to gE after RZV (data not shown). In multivariate analysis, both antibody and cellular responses remained significantly higher in the TN vs BTKi group (odds ratio 4.26 [95% CI, 1.80-10.10], $P = .001$; odds ratio 3.66 [95% CI, 1.47-9.14], $P = .005$), after controlling for demographics and other baseline characteristics (supplement 3).

Long-term humoral response was assessed in 26 patients after 12 months and 12 patients after 24 months. The median follow-up in the long-term follow-up cohort was 12 months (interquartile range 12, 24). Among 26 patients, 21 (80.8%) received BTKi and 5 (19.2%) were patients with TN CLL. Eleven (42.3%) patients had a positive serologic response at 6 months (≥fourfold rise in anti-gE). Antibody titers did not significantly change between 6, 12, and 24

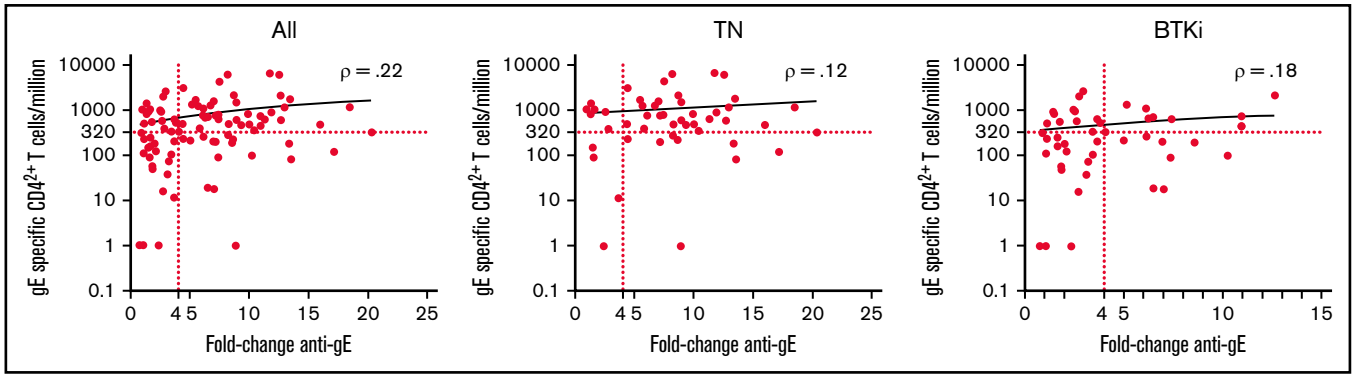

Figure 3. Paired anti-gE IgG fold-change (x-axis) and gE-specfic $CD4^{2+}$ cells per million $CD4^{+}$ T cells (y-axis) at 6 months in paired samples. The dashed red lines represent the response threshold (≥fourfold rise in anti-gE titer) or >320 gE-specific $CD4^{2+}$ T cells/million $CD4^{+}$ T cells).

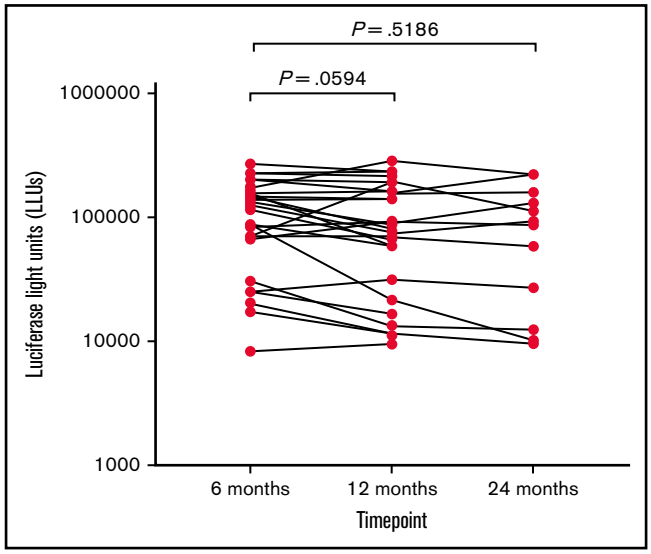

Figure 4. Serologic anti-gE titers compared between 6, 12, and 24 months. Bars indicate geometric mean and 95% CI.

months after vaccination across the entire patient population, within each cohort (TN and BTKi), and among responders and nonresponders (Figure 4). There was no significant difference in anti-gE titer changes at 6, 12, and 24 months in TN and BTKi cohorts or in responders and nonresponders at 6 months (all $P > .05$).

Safety

The most frequent local and systemic AEs were injection site pain (97.4%), injection site reaction (70.7%), headache (51.7%), and generalized myalgias (51.7%) (Table 5). Most AEs were grade 1-2, and all AEs resolved or returned to baseline within 7 days of vaccine administration. Grade 3 AEs occurred in 17 (14.7%) patients. No grade 4 or higher AEs or treatment-related serious AEs occurred, and there was only 1 serious AE (pneumonia) that was deemed unrelated to RZV and eventually resolved. There was 1 case of clinically suspected grade 2 herpes zoster reactivation that occurred 14 days after the second vaccination. VZV polymerase chain reaction or direct fluorescent antibody testing was not performed. Antiviral therapy led to complete resolution and the case was deemed unrelated to vaccination. The patient developed a positive antibody response, but no cellular response 3 months after the second vaccination. There were no cases of postherpetic neuralgia during the study period.

Discussion

This study confirms the safety and reduced immunogenicity of the RZV vaccine in patients with CLL who are untreated or receiving treatment with a BTKi. The vaccine antibody response rate in the TN CLL cohort (76.8%) is similar compared with responses observed in other hematologic malignancies (80.4%),[6] yet decreased compared with the general population (97.8%).[19] Conversely, BTKi therapy significantly impairs vaccine responses, eliciting humoral responses in only 40.0% of patients. The median fold-change of anti-gE titers after the second vaccine dose was attenuated in both the TN (7.76) and BTKi (3.31) cohort compared with expected fold-change in gE in the general population (39.1).[19] It is conceivable the lower antibody titers confer lower rates of protection against infection; however, it is currently unknown what level of gE fold-change provides optimal protection against RZV infection. Notably, in the interim analysis of this study, there was no statistically significant difference in vaccine antibody responses between TN- vs BTKi-treated patients (41.5% vs 59.1%; $P = .2$).[7] The analysis of the complete study population showed a larger difference between vaccine responses in the TN and BTKi groups that was statistically significant (76.8% vs 40.0%; $P = .0002$). Long-term antibody titers following RZV vaccination remained stable for at least 2 years after vaccination. These findings differ from immunogenicity patterns among the general population[20]; adults ≥60 years of age receiving RZV vaccination achieve peak antibody titers 1 to 2 months after

Table 5. Adverse events

	Grade 1	Grade 2	Grade 3	Grade 4 or 5	Any grade
Subjects with at least 1 AE	28 (24.1%)	69 (59.5%)	17 (14.7%)	0 (0%)	114 (98.3%)
Injection site pain	58 (50%)	48 (41.4%)	7 (6.0%)	0 (0%)	113 (97.4%)
Injection site reaction	28 (24.1%)	54 (46.6%)	0 (0%)	0 (0%)	82 (70.7%)
Headache	44 (37.9%)	10 (8.6%)	6 (5.2%)	0 (0%)	60 (51.7%)
Myalgia	39 (33.6%)	17 (14.7%)	4 (3.4%)	0 (0%)	60 (51.7%)
Fatigue	34 (29.3%)	20 (17.2%)	4 (3.4%)	0 (0%)	58 (50%)
Flulike symptoms	18 (15.5%)	14 (12.1%)	3 (2.6%)	0 (0%)	35 (30.2%)
Chills	20 (17.2%)	3 (2.6%)	0 (0%)	0 (0%)	23 (19.8%)
Nausea	20 (17.2%)	1 (.9%)	0 (0%)	0 (0%)	21 (18.1%)
Rash, maculopapular	10 (8.6%)	0 (0%)	0 (0%)	0 (0%)	10 (8.6%)
Vaccination site lymphadenopathy	9 (7.8%)	0 (0%)	0 (0%)	0 (0%)	9 (7.8%)
Fever	6 (5.2%)	1 (.9%)	1 (.9%)	0 (0%)	8 (6.9%)
Lung infection	0 (0%)	0 (0%)	1 (.9%)	0 (0%)	1 (.9%)
Syncope	0 (0%)	0 (0%)	1 (.9%)	0 (0%)	1 (.9%)

All treatment-related AEs (TRAEs) occurring at a frequency ≥5% and any grade ≥3 treatment-emergent AEs are listed. When multiple incidents of the same AE are reported, only the AE with the highest grade is counted.

vaccination, with a gradual decrease in anti-gE titers over time, achieving a plateau 4 years after vaccination with long-term persistence up to 10 years after vaccination.

Cellular and humoral response rates following RZV were similar within the TN cohort (76.8% and 70.0%) and the BTKi cohort (40.0% and 41.3%). Furthermore, there was no difference in cellular and humoral response rates in patients treated with either ibrutinib or acalabrutinib. Given the increased selectivity of acalabrutinib, this finding suggests that vaccine responses are affected by the on-target inhibition of BTK rather than off-target effects.[21] Approximately two-thirds of subjects (69.1%) had both a humoral and cellular response. Notably, more than one-third (39.0%) mounted a T-cell response following vaccination despite having a negative antibody response. In the general population, antibody and cellular immune responses appear coordinated.[22] The agreement between the antibody and cellular response in CLL is only partial, possibly because of cancer- and therapy-mediated impairment of B- and T-cell signaling. The clinical significance of a positive T-cell response without a concomitant antibody response requires further study; however, T-cell responses, not antibody responses, have been shown to correlate with reduced severity of zoster infection.[10,23] We did not find a significant correlation between antibody or cellular responses and age or immunoglobulin levels. This finding is consistent with other RZV vaccine studies in patients with CLL,[11,12] yet COVID-19 vaccine responses in CLL improve with younger age and immunoglobulin G levels ≥550 mg/dL.[15]

The results of cellular immunity reported here are similar to those obtained in another, smaller, RZV vaccine study by Muchtar et al.[11] That study included 22 TN subjects and 19 BTKi-treated subjects with a 52.0% and 28% T-cell immunogenicity rate, respectively. Notably, a different study by Zent et al found higher humoral and cellular vaccine responses in BTKi-treated patients[12]; 32 patients with CLL (n = 22) and lymphoplasmacytic lymphoma (n = 10) treated with ≥3 months of BTKi had a 75.0% humoral response rates and a 78.1% cellular response rate.[12] Possible explanations for the discrepancy between response rates in this report and the study conducted by Zent et al are that the latter study had (1) smaller and variable patient population because of the inclusion of patients with lymphoplasmacytic lymphoma, (2) inclusion of patients who may be less immunocompromised (exclusion of patients with lymphocyte count $>20 \times 10^9$/L vs no exclusion based on lymphocyte count in this study), (3) less restrictive cellular response criteria (≥twofold increases in the frequency of activated $CD4^+$ T-cell clusters vs ≥twofold rise over baseline and ≥320 net gE-specific $CD4^{2+}$ cells per million $CD4^+$ T cells in this study), and (4) lack of $CD19^+$ B-cell depletion, potentially making use of antigen-presenting capabilities and immunogenicity of residual B cells (this study performed ex vivo $CD19^+$ depletion). Consistent with our findings, other studies investigating the COVID-19 vaccine confirmed diminished humoral response rates in patients with CLL treated with a BTKi.[15] Taken together, BTKi therapy negatively affects both humoral and cellular vaccine responses in CLL.

Finally, the safety of RZV administration in patients with CLL is also reassuring. The rates of AEs in patients with CLL receiving RZV were comparable to those observed in the general population.[19] The lack of adverse safety signal paired with decreased immunogenicity found in this population highlights the need to include patients with CLL treated with novel therapies in large vaccine trials. Historically, some studies have excluded CLL and other B-cell malignancies from primary analyses because of concerns of futility and decreased immune responses.[6] We demonstrate that a significant proportion of patients with CLL mounted immune responses to RZV and may derive clinical protection from other vaccinations.

Although the difference in immunogenicity between the TN and BTKi groups is striking and observed in other vaccine studies,[15,24,25] it is uncertain if and how BTKis directly inhibit immune responses. BTKi therapy may inherently reduce vaccine immunogenicity via disruption of B-cell receptor signaling and inhibition of antigen-presenting cells.[26] Conversely, TN patients with CLL could be more immunocompetent because of less advanced disease, permitting more effective immune responses. This study did not include patients treated with other commonly used CLL regimens, including B-cell lymphoma 2 inhibitors (eg, venetoclax) and or anti-CD20 therapy (eg, rituximab). Anti-CD20 therapy is associated with a marked reduction in immunogenicity, whereas the effect of B-cell lymphoma 2 inhibitors on vaccine responses remains uncertain because of the frequent use of anti-CD20 treatment in combination with venetoclax.[15] A previous study in the general population observed peak response rates 1 month after the second vaccination, with a gradual decline in titers over time.[19] The fold increase in anti-gE decreased from 39.1 at 1 month after vaccination to 8.3 at 36 months after the second vaccine. This study measured responses 3 months after the second vaccination; therefore, it is possible that we provide an underestimation of the peak response rate. However, long-term assessment of titers may be a more relevant measure of immunity, as demonstrated by the stability of antibody titers up to 24 months after vaccination.

In light of the ongoing COVID-19 pandemic, there are continued efforts toward improving vaccine response rates in at-risk populations. Possible strategies involve booster/revaccination at regular intervals or brief interruption of BTKi therapy around the time of vaccination in clinically stable patients. Further studies are required to confirm the clinical impact and assess the possible adverse effects of such strategies. Encouragingly, antibody titers persisted for at least 2 years after vaccination in our study, suggesting sustained benefits after vaccination. In summary, RZV is safe in patients with CLL and can induce both humoral and cellular immune responses in many patients. BTKi treatment was associated with lower humoral and cellular vaccine responses compared with TN patients. All patients with CLL should receive vaccination against novel antigens, ideally before starting a BTKi.

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Authorship

Contribution: C.P., C.S., A.W., and D.M.K. conceived and designed the analysis and wrote the manuscript; I.E.A. conceived and designed the analysis; X.T. performed data analysis; P.N., E.M., and S.S. collected data and coordinated the study; J.L. and J.S. collected data; and D.M.K., K.J.L., C.L.M., J.I.C., and M.A.A. contributed analysis tools and performed data analysis.

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