Systemic and mucosal adaptive immunity to SARS-CoV-2 during the Omicron wave in patients with chronic lymphocytic leukemia

The COVID-19 pandemic has significantly impacted patients with chronic lymphocytic leukemia (CLL),[1] with many failing to seroconvert[2] or mediate variable T-cell immunity[3] after mRNA vaccination. The emergence of the B1.1.529 (Omicron) variant of SARS-CoV-2 has altered the development of the COVID-19 pandemic due to its less severe clinical course and associated reduced risk of hospitalization.[4] However, the impact of Omicron on immunosuppressed subgroups, such as patients who have received CD20 monoclonal antibodies (mAb)[5] remains uncertain. Moreover, the observed decrease in severe disease cases within the general population may be influenced by the high number of infected individuals.[6]

In addition to the systemic immunoglobulin (Ig) G response, SARS-CoV-2 infection induces production of specific secretory IgA in mucosal secretions from local plasma cells, and serum IgA from plasma cells homing to the bone marrow.[7] Whether this occurs after Omicron infection in patients with hematological or solid cancer remains elusive. We report here on serological, cellular, and mucosal immune response in a cohort of patients with CLL diagnosed with symptomatic SARS-CoV-2 infection during the Omicron BA.1 and BA.2 wave.

Twenty-six patients with CLL who had symptoms of COVID-19 and tested positive for SARS-CoV-2 between January 9, 2022 and April 29, 2022, were included. Ninety-nine percent of all sequenced SARS-CoV-2 samples in Sweden taken on January 17, 2022 or later were Omicron variants.[8] Patients diagnosed earlier than January 17, 2023 were only included if viral sequencing confirmed Omicron. The national ethics authority approved the study. Written informed consent was obtained from each patient before samples were obtained.

The clinical characteristics are summarized in Table 1. Three patients had had a previous polymerase chain reaction (PCR)-verified SARS-CoV-2 infection at a median time of 20 months earlier (range, 13-21). Their immunological outcomes were similar to those who had Omicron as their first-time infection (*data not shown*). Patients had either early-stage, untreated CLL (n=11) or had ongoing CLL treatment (n=12), either Burton tyrosine kinase inhibitor (BTKi) therapy (n=11) or venetoclax + CD20 mAb (n=1). Four patients paused their BTKi treatment for a few days during the infection, and their immunological outcomes were similar to those who continued (*data not shown*). Five additional patients had completed various prior CLL therapies (including CD20 mAb) at a median time of 26 months before infection (range, 8-74). Five patients had ongoing immunoglobulin supplemental treatment (IVIG).

Total Ab levels against SARS-CoV-2 Spike receptor-binding domain (RBD) protein were analyzed in 14 of 26 patients at the time point when they had just been diagnosed with active, symptomatic COVID-19 infection, using Elecsys® anti-SARS-CoV-2 S immunoassay (Roche Diagnostics) (positive test was defined as >0.8 U/mL, patients with IVIG treatment were not included). Fifty percent (7/14) were seronegative, and of these, four had received a third vaccine dose 2-4 months before the infection and three had received 1-2 doses 9-11 months before. Two to three weeks after clinical recovery, a positive Elecsys® total anti-RBD test was noted in 81% of analyzed patients (13/16, 1 missing sample, 9 samples were excluded from analysis due to treatment with IVIG or the anti-SARS-CoV-2 mAb sotrovimab).

We next used the V-PLEX Panel 25 assay (Meso Scale Discovery[9]) to differentiate IgG and IgA reactivities against ten different SARS-CoV-2 Spike variants in serum (n=24, 2 missing samples) respectively in saliva (n=25, 1 missing sample) from the convalescence follow-up. The serum was analyzed according to the manufacturer's instructions, and saliva collection has been described elsewhere.[10] Cutoff levels for positive saliva reactivity was defined for each antigen using pre-pandemic samples from healthy donors. Serum IgG was not analyzed in samples from patients who had received IVIG or sotrovimab treatment (n=9). Results against all SARS-CoV-2 variants are shown in the *Online Supplementary Figure S1*.

Positive IgG levels against the Wuhan-Hu-1 (wild-type) SARS-CoV-2 variant (defined by the manufacturer as >1,960 AU/mL) were noted in all but one convalescent serum sample (Figure 1A). Generally, IgG reactivity against the three main variants (wild-type, Omicron BA.1 and Omicron BA.2 variants) varied substantially between individuals, and no significant differences were noted between the CLL treatment subgroups. Congruent with the serum findings, IgG reactivity against any SARS-CoV-2 Spike variant was observed in 88% of convalescent saliva samples (22/25; *Online Supplementary Figure S1C*), without difference in frequency or magnitude between the CLL treatment subgroups when comparing reactivity to the three main variants (Figure 1B). In contrast to the IgG reactivity, the serum IgA (i.e., mucosa-derived) responses to BA.2 Spike were significantly lower in BTKi/BCL-2i treated patients than in early-stage untreated patients (P=0.012) with a similar trend for responses against the wild-type variant (P=0.051) (Figure 1C).

Table 1. Clinical characteristics of patients with chronic lymphocytic leukemia (N=26) at the time of SARS-CoV-2 Omicron infection.

	Early-stage untreated N=11	Previously treated[a] N=5	Ongoing BTKi/BCL2i[b] N=12	Entire cohort N=26
Median age in years (range)	71 (53-82)	75 (63-87)	70.5 (42-82)	70.5 (42-87)
Male/Female, N	5/6	3/2	10/2	17/9
Time in years since CLL diagnosis, median (range)	3 (1-16)	8 (4-24)	9.5 (0-19)	7.5 (0-24)
CLL stage (Rai), N (%) 0 I-II III-IV	 8 (73) 2 (18) 1 (9)	 3 (60) 0 2 (40)	 11 (92) 1 (8) 0	 20 (77) 3 (12) 3 (12)
CLL remission status (iwCLL), 24 evaluated, N (%) PR/CR SD PD	 - 7/9 (78) 2/9 (22)	 - 3 (60) 2 (40)	 12 (100) 0 0	 12/24 (50) 9/24 (38) 3/24 (13)
Ongoing Ig supplement, N (%)	0	1 (20)	4 (33))	5 (19)
Comorbidities, N (%) Hypertension COPD/asthma Diabetes	 7 (64) 2 (18)) 3 (27)	 4 (80) 0) 1 (20)	 5 (42) 2 (17) 2 (17)	 14 (54) 4 (15) 5 (19)
Vaccination status[c], N (%) 4 doses 3 doses 2 doses 1 dose Unvaccinated	 2 (18) 8 (73) 1 (9) 0 0	 1 (20) 2 (40) 1 (20) 0 1 (20)	 1 (8) 7 (58) 3 (25) 1 (8) 0	 3 (12) 16 (62) 5 (19) 1 (4) 1 (4)
Time in months since last vaccine dose, median (range)	2.1 (1-6.6)	2.6 (0.5-9)	3.4 (1-11)	2.7 (0.5-11)
Omicron variant, N=12 of sequenced samples, N BA.1/BA.2	 Unknown	 4/0	 3/5	 7/5
Admitted to hospital, N (%)	2 (18)	4 (80)	5 (42)	9 (35)
Length in days of hospital stay, median (range)	12 (8-16)	7.5 (1-18)	5 (3-9)	7 (1-18)
Omicron treatment, N (%) Supplementary oxygen Corticosteroids Sotrovimab Remdesivir	 1 (9) 2 (18) 1 (9) 1 (9)	 3 (60) 3 (60) 3 (60)) 2 (40)	 2 (17) 1 (8) 5 (42) 4 (33)	 5 (19) 4 (15) 8 (31) 6 (23)
Secondary bacterial infection, N (%)	2 (18)	4 (80)	1 (8)	7 (27)

[a]All >6 months ago. Chemoimmunotherapy (N=3), ibrutinib (N=1), rituximab (N=2). [b]Bruton´s tyrosine kinase inhibitor (BTKi) (N=14) or venetoclax (BCL2i) (N=2). [c]Six patients received *Vaxzevria* (AstraZeneca) at dose 1 and 2, all other were mRNA vaccinations (*Comirnaty*, Pfizer BioNTech or *Spikevax*, Moderna). CLL: chronic lymphocytic leukemia; iwCLL: International Workshop on Chronic Lymphocytic leukemia; PR: partial remission; CR: complete remission; SD: stable disease; PD: progressive disease; COPD: chronic obstructive pulmonary disease. Ig: immunoglobulin.

Furthermore, salivary Spike-specific IgA against any variant was detected only in 40% (10/25; *Online Supplementary Figure S1D*) of patients. In line with the serum findings, IgA response was more rarely detected in saliva in patients with ongoing BTKi/BCL-2i therapy compared to early-stage untreated patients (2/12 *vs.* 6/9; *P*=0.032). The magnitude of the IgA salivary response was also significantly lower in BTKi/BCL-2i treated patients than the early-stage untreated patients when comparing the three main variants separately (Figure 1D; wild-type *P*=0.010; BA.1 *P*=0.016; BA.2 *P*=0.038). The ability of the convalescence sera to block Spike-protein binding to ACE2, a measure of viral neutralization capacity,[11] was measured in 15 samples (9 samples were excluded due to sotrovimab or IVIG treatment) using the V-PLEX SARS-CoV-2 Panel 25. Fifty-three percent (8/15) were able to neutralize at least one Spike variant to 50% inhibition or higher (*Online Supplementary Figure S2*). Conversely, only 16% of saliva samples (4/25; 2 early-stage untreated, 1 previously treated, and 1 with ongoing BTKi/BCL-2i therapy) were able to neutralize at least one Spike variant (*Online Supplementary Figure S2*). The neutralization magnitude did not differ significantly between the patient subgroups (*data not shown*).

The correlation between IgG and IgA levels and the cor-

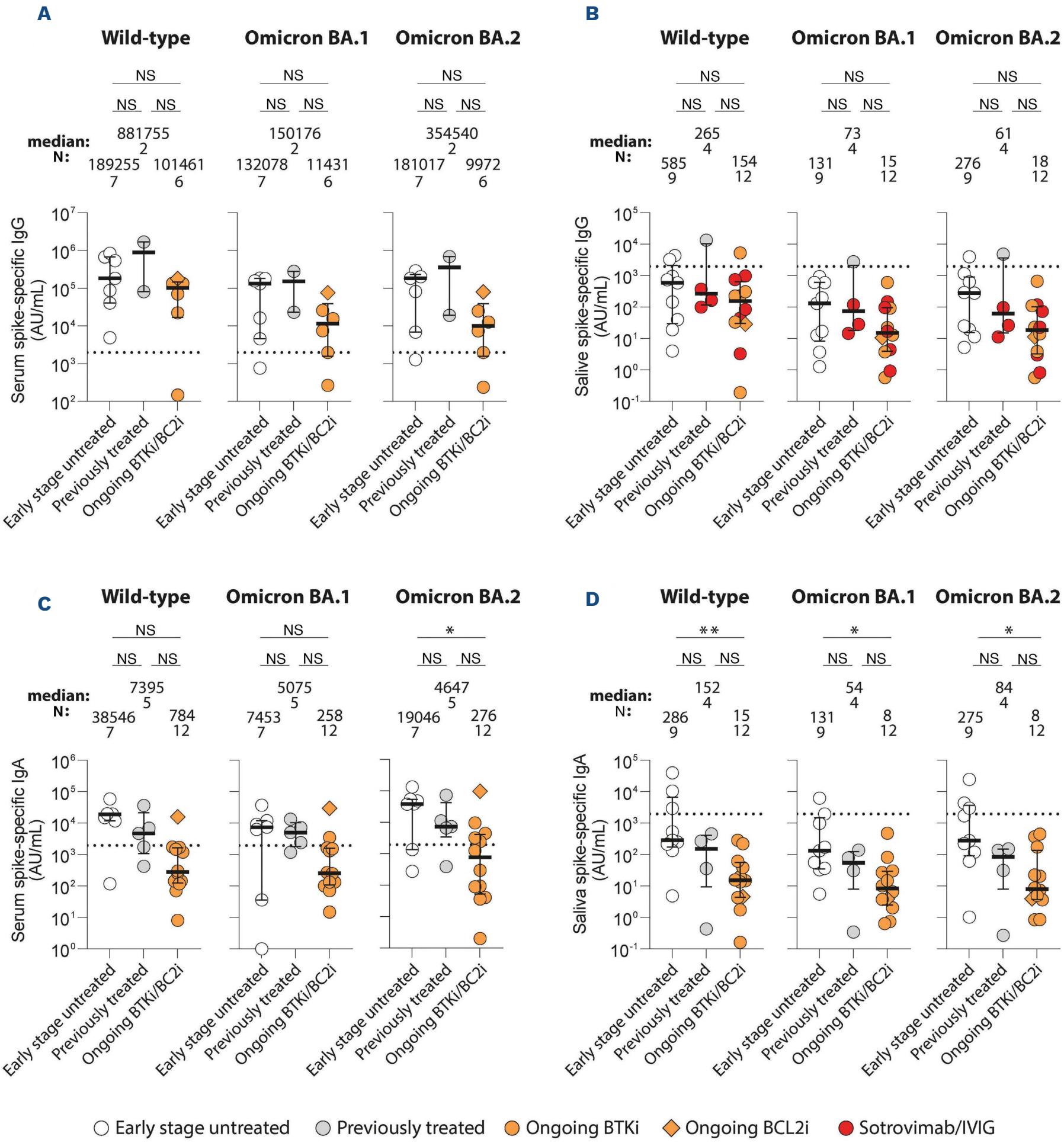

Figure 1. Spike-specific antibodies in serum and saliva after clinical recovery from Omicron infection. Anti-Spike immunoglobuln (Ig) G in convalescent sera (A) and saliva (B) specific for SARS-CoV-2 wild-type, Omicron BA.1, and Omicron BA.2. Patients who had received IVIG or sotrovimab are excluded from the serum analyses and highlighted (red) in the saliva panel (B). The corresponding anti-Spike IgA levels are shown in (C) (serum) and (D) (saliva). Cutoff levels (dotted lines) for positive responses against wild-type in serum were determined by the manufacturer (1,960 AU/mL) and against all antigens in saliva using prepandemic saliva samples (defined as the mean plus 6x standard deviation of the intensity signals of 27 negative prepandemic saliva samples) and were as follows: anti-wild-type IgG: 4.01 AU/mL; anti-BA.1 IgG: 4.98 AU/mL; anti-BA.2 IgG: 7.33 AU/mL; anti-wild-type IgA: 226.72 AU/mL; anti-BA.1 IgA: 81.77 AU/mL; anti-BA.2 IgA: 203.18 AU/mL. Median and interquartile range are indicated in the panels. Statistics was assessed with non-parametric Kruskal-Wallis' test with Dunn's multiple comparison correction. *P<0.05, **P<0.01 and NS P>0.05: not statistically significant.

responding neutralization capacity was stronger in serum than in saliva, and more pronounced for the wild-type variant compared to BA.1 (*Online Supplementary Figure S3*). The serum and salivary neutralization capacity of Omicron BA.2 was generally low, and correlation with corresponding Ab levels was hence not done.

Next, we measured SARS-CoV-2-specific T-cell responses to wild-type and Omicron Spike-specific peptides using an AIM assay (Figure 2A), as previously described.[12] PBMC were collected after clinical recovery from 22 patients (8 with untreated early-stage CLL, 4 previously treated, 9 with ongoing BTKi, and 1 with venetoclax + CD20 mAb treatment). Eight otherwise healthy and previously vaccinated individuals who had recovered from Omicron infection served as controls. Specific CD4$^+$ T cells against wild-type Spike were detected in 95% (21/22) of patients and against Omicron BA.1 Spike in 91% (20/22) (Figure 2B). Marginally lower frequencies of Spike-specific CD8$^+$ T cells (Figure 2C) were observed, as 77% (17/22) and 73% (16/22) of patients had a response against wild-type and Omicron BA.1, respectively. No significant correlation was found between T-cell responses and serum or saliva reactivities, and also seronegative convalescents had measurable T-cell responses (*data not shown*). The magnitudes of the T-cell responses

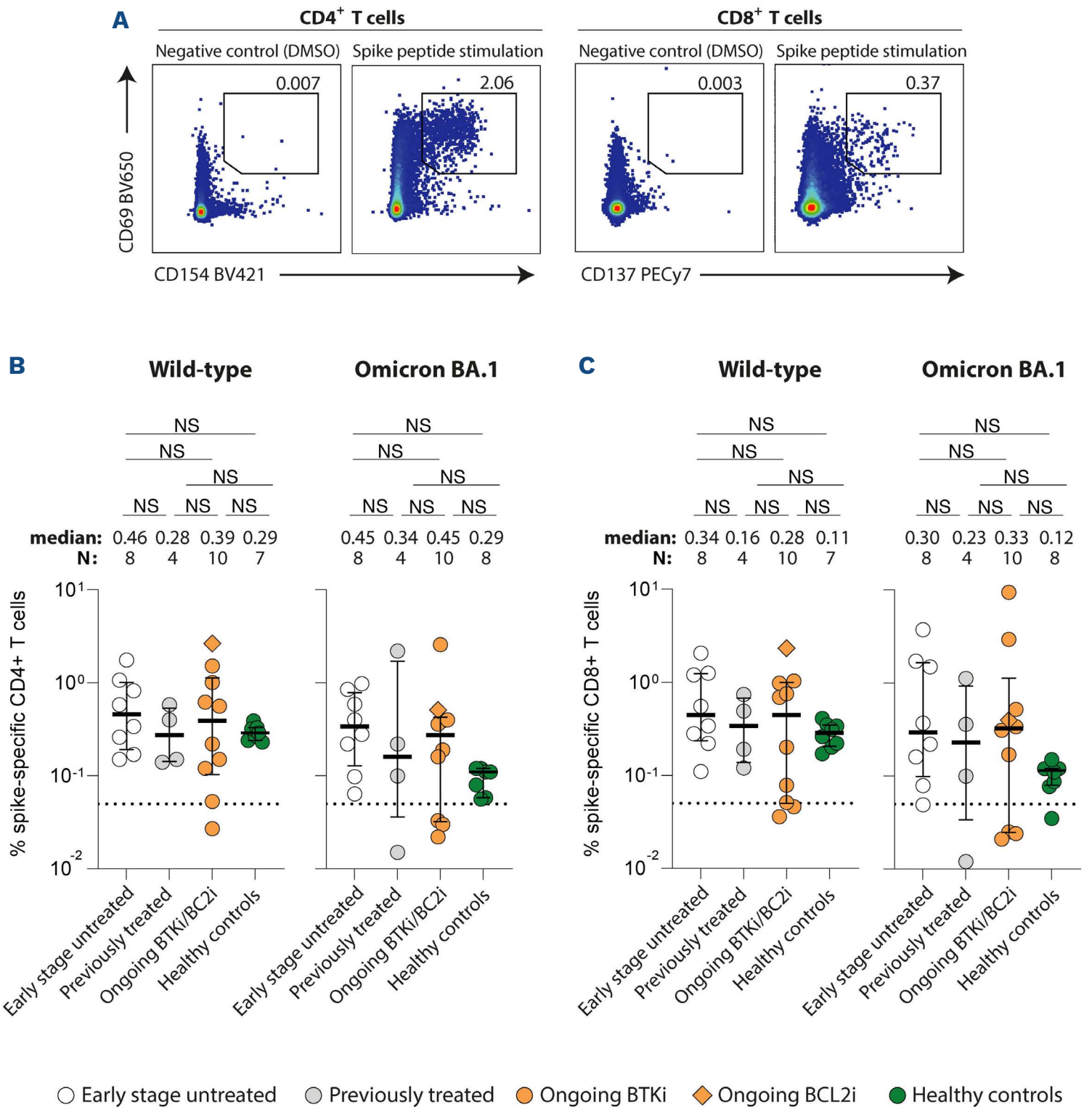

Figure 2. SARS-CoV-2 reactive T cells in chronic lymphocytic leukemia patients and healthy controls after clinical recovery from Omicron infection. (A) Representative flow cytometry plot of antigen-specific CD4$^+$ (CD69$^+$CD154$^+$) and CD8$^+$ (CD69$^+$CD137$^+$) T cells after peptide stimulation. Frequencies of Spike-specific CD4$^+$ (B) and CD8$^+$ (C) T cells against SARS-CoV-2 wild-type and Omicron BA.1 peptides. A positive response was defined with a cutoff level of 0.05%. Median and interquartile range are indicated in the panels. Statistics was assessed with non-parametric Kruskal-Wallis' test with Dunn's multiple comparison correction. NS P>0.05: not statistically significant. DMSO: dimethyl sulfoxide.

were similar in all CLL treatment subgroups and comparable to those of the healthy individuals (Figures 2B, C).
Taken together, many patients mounted high post-infection IgG levels and T-cell responses. Notably, the T-cell responses were similar to those of healthy donors, also in patients with B-cell inhibiting therapy or low or absent convalescent Ab levels, which is most likely of clinical importance.[12] However, we found an impaired IgA reactivity against all three virus variants in patients with ongoing BTKi/BCL-2i therapy in saliva, with a similar trend in serum, suggesting a previously not yet described negative effect of precision B-cell inhibiting treatment on mucosal immunity. Whether this is related to impaired mucosal memory B cells[13] remains to be shown. Healthy individuals have significantly better protection against SARS-CoV-2 infection with higher mucosal IgA levels,[14] and further studies are required on how the decreased IgA levels and generally low neutralization capacity of saliva Ab affect the risk of re-infection, particularly in BTKi-treated individuals. Notably, a significant reduction in the risk of grade 3-4 bacterial infections, mainly pneumonia, has been reported when the administration of BTKi is temporarily ceased in patients with CLL.[15] This observation suggests a more widespread impairment of mucosal immunity post-BTKi, which also extends to other pathogens.
The major limitations of our study are the small number of included patients and the heterogeneity of both previous CLL treatment, number of vaccine doses and antiviral treatment, including short-term use of corticosteroids, which might have impacted the immunological response. Also, the use of immunoglobulin treatment limited the number of IgG analyses.
We provide a comprehensive analysis of both systemic and mucosal immunity to ten SARS-CoV-2 variants after Omicron infection in patients with CLL. Our data indicate that patients on BTKi/BCL-2i therapy exhibit compromised mucosal immunity, potentially increasing the susceptibility of this already vulnerable population to recurrent episodes of SARS-CoV-2 infection.

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Disclosures

MB is a consultant and has received honoraria from MSD, Pfizer, Mabtech, BMS, and Oxford Immunotec. AS is a consultant for Gritstone Bio, Flow Pharma, Moderna, AstraZeneca, Qiagen, Fortress, Gilead, Sanofi, Merck, RiverVest, MedaCorp, Turnstone, NA Vaccine Institute, Emervax, Gerson Lehrman Group and Guggenheim. La Jolla Institute for Immunology has filed for patent protection for various aspects of T-cell epitope and vaccine design work. All other authors have no conflicts of interest to disclose.

Contributions

HMIS, LB, AÖ, GB, SA, MSC, HGL, and MB contributed to the conceptualization, funding acquisition, and discussion of data. DW, JW, YG, KH, PC, MÅ, and SM performed experiments and analyzed data. LB, HMIS, CK, LH, and AÖ recruited study participants, conducted the management of participants during the study, and analyzed data. AG and AS provided peptide pools to measure the Spike-specific T-cell responses. OB provided information on

sequencing. HMIS, LB, DW, KH, MSC, AÖ, HGL, and MB wrote the original draft of the manuscript. All authors reviewed and edited revisions of the manuscript and had final responsibility for the decision to submit for publication.

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Data-sharing statement

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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