SARS-CoV-2-specific cellular response following third COVID-19 vaccination in patients with chronic lymphocytic leukemia

With great interest we read the study published by Blixt *et al.* showing that compared to healthy controls (HC), half as many of chronic lymphocytic leukemia (CLL) patients developed a T-cell response after two COVID-19 vaccine doses.[1] Effects of a third vaccine dose on T cells in CLL patients is yet unknown, while approximately 20% fail achieving a humoral immune response.[2] In this prospective cohort study we investigated the interplay of humoral and cellular response and report follow-up data of CLL patients 31 days (range, 19-94 days) after third vaccination (V3).[3]

Blood samples of CLL registry (clinicaltrials gov. Identifier: NCT02863692) patients were evaluated after three COVID-19 vaccinations. Six of the initially 21 patients[3] were included in the analyses, three with homologous and three with heterologous vaccination schedule (mean interval between vaccination 2 [V2] and V3 163 days; minimum 117 days and maximum 189 apart). Four vaccinated health care workers served as HC (mean interval between V2 and V3 266 days; range, 254-291 days). Both studies were approved by the local ethics committee. Patient and disease characteristics as well as vaccination schedules are summarized in Table 1.

SARS-CoV-2 spike receptor binding domain (RBD)-specific immunoglobulin G (IgG) antibodies, determined using the Alinity ci SARS-CoV-2 IgG II Quant assay (Abbott), were detectable in four of six (66.7%) CLL patients after compared to two of six (33.3%) before booster vaccination (Figure 1A), cut-off ≥7.1 BAU/mL. In the one individual with detectable RBD-specific IgG after V2, V3 resulted in increased levels. In another individual, the V3 raised the IgG titer to similar levels as seen shortly after V2 (Figure 1B and C). Detectable neutralizing serum activity, determined by a lentivirus-based pseudovirus neutralization assay against the Wu01 strain of SARS-CoV-2 was limited to the two individuals with the highest levels of RBD-binding IgG (Figure 1D).

Peripheral blood mononuclear cells (PBMC) were used for SARS-CoV-2 spike-specific T-cell analyses (Human IFNγ ELISpot^{PLUS} [ALP] kit [Mabtech]). Results are reported as spot-forming cells (SFC) per million PBMC. A SARS-CoV-2 peptide pool (15-mers overlapping by 11 amino acids which stimulate responses mediated by both CD4 + and CD8 + T cells) spanning the entire spike protein was used for measuring T-cell responses. The median number of SARS-CoV-2 spike-specific T cells in the CLL cohort after V2 BNT162b was 31 SFC (interquartile range [IQR], 4.0-96.0) (Figure 2A). The response after V2 in the here described subgroup was significantly lower (1.7 SFC; IQR, 0.0-3.8 but increased to 8 SFC; IQR, 5.7-21.3) after booster vaccination. Overall, four of six (66.7%) showed a detectable increase of T-cell activity and two a decrease (Figure 2B). In comparison, T-cell responses in HC remained above the cut-off in 100% (4/4), but did not increase further.

Of the included patients, all received either B-cell-depleting (anti-CD20 monoclonal antibodies) or -directed (bruton tyrosine kinase inhibitors) treatment within 6 months prior to V3. Despite B-cell-affecting treatment, the majority (4/6) showed an increase of serum IgG (Figure 1C). Patients under B-cell-depleting treatment (2/6) mounted low levels of IgG antibodies after boost that did not result in detectable neutralizing serum activity (Table 1). Patients without detectable T cells prior to boost that received a heterologous booster immunization showed an increase in T-cell response. In contrast, homologous booster led to an increase in only one of three patients and did not show an effect on the remaining two patients (Figure 2B). A discordant immune response with T cell, but lacking humoral response was seen in two of six patients, indicating that cellular protection may be generated, probably in patients with lesser extent of CLL-associated T-cell exhaustion, whereas treatment-associated B-cell impairment may not be overcome.

In conclusion, we report an increase of vaccine-induced cellular and humoral immune responses in CLL patients by a V3 COVID-19 vaccination.

Recent data showed a significant increased humoral response after COVID-19 vaccination, but less pronounced enhancement of the cellular response in healthy individuals, likely to be dependent on the specific booster vaccine.[4-6] Our data from the HC cohort – all vaccinated with a homologous BNT162b2 dose – confirm these findings and show a stable, but not relevantly increased T-cell response. As already shown for rheumatologic and solid organ transplant patients, this may not generally be the case for immunocompromised patients.[7,8]

We here report an increase of the humoral response in CLL patients after COVID-19 V3 despite B-cell-depleting treatment, as reported elsewhere,[9] and in addition, an increase of the cellular response in four of six patients.

Our data show that V3 enhances IgG response in CLL patients, also in those that lacked detectable IgG after V2.

Table 1. Patient characteristics and outcomes versus healthy controls.

Patient	Sex	Age in years	Vaccine (Prime)	Vaccine (Boost)	Days of sampling after booster	After V2			After V3			B-cell-depleting therapy	B-cell-directed therapy	State of disease
						IgG**	Serum ID_{50}***	T cells*	IgG**	Serum ID_{50}***	T cells*			
1	M	78	BNT/BNT	BNT	13	33	<10	4	42,9	44	25	No	Yes	PR
2	M	78	BNT/BNT	AZD	31	neg.	<10	0	neg.	<10	9	No	Yes	PR
3	M	76	BNT/BNT	BNT	28	2.225	1.206	3	10.999	19.214	0	No	Yes	PR
4	M	71	BNT/BNT	AD	63	neg.	<10	0	8,3	<10	7	Yes	Yes	CR
5	F	73	BNT/BNT	AD	36	neg.	<10	0	11,8	<10	81	Yes	No	CR
6	M	71	BNT/BNT	BNT	31	neg.	<10	23	neg.	<10	5	No	Yes	PD

Healthy control	Sex	Age in years	Vaccine (Prime)	Vaccine (Boost)	Days of sampling after booster	After V2	After V3
						T cells*	T cells*
1	F	25	BNT/BNT	BNT	28	165.3	202.7
2	M	30	BNT/BNT	BNT	28	381.3	404
3	F	38	BNT/BNT	BNT	37	692	428
4	F	49	BNT/BNT	BNT	27	116	77.3

*S-specific T cells (spot-forming cells/10^6 peripheral blood mononuclear cells); **RBD-specific IgG (BAU/mL); ***neutralization activity (serum ID50 Wu01 Pseudovirus). V2: second vaccination; V3: third vaccination; IgG: immunoglobulin G; F: female; M: male; ID_{50}: infectie dose; AD: Ad26.COV2, AZ: AZD1222, BNT: BNT162b2; CR: complete remission; PD: progressive disease; PR: partial remission; neg: negative.

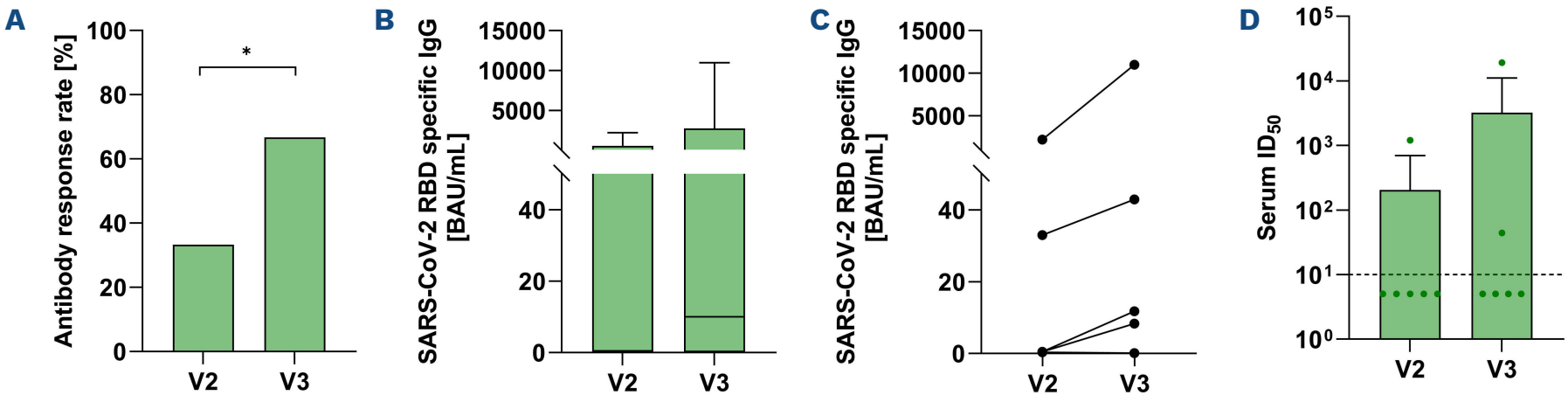

Figure 1. Humoral immune responses after COVID-19 vaccination (A) Antibody response rate in chronic lymphocytic leukemia (CLL) patients after second (V2) and after third (V3) vaccination. (B) SARS-CoV-2 spike receptor binding domain (RBD)-specific immunoglobulin G (IgG) in CLL patients after V2 and V3 (median 10.05 BAU/ml, range 0.1-10,998.6) measured by chemiluminescent microparticle immunoassay. (C) Individual course of IgG anti-bodies in CLL patients after V2 and V3. (D) Serum neutralizing activity (50% inhibitory serum dilution) determined in a pseudovirus neutralizing assay against the Wu-01 pseudovirus strain. Bars indicating geometric mean ID_{50} with 95% confidence intervals. Dashed line indicates limit of detection (LOD, 10). Samples with no detectable neutralization (ID_{50} <10) were plotted with an ID_{50} of 5 (1/2 LOD) for graphical representation.

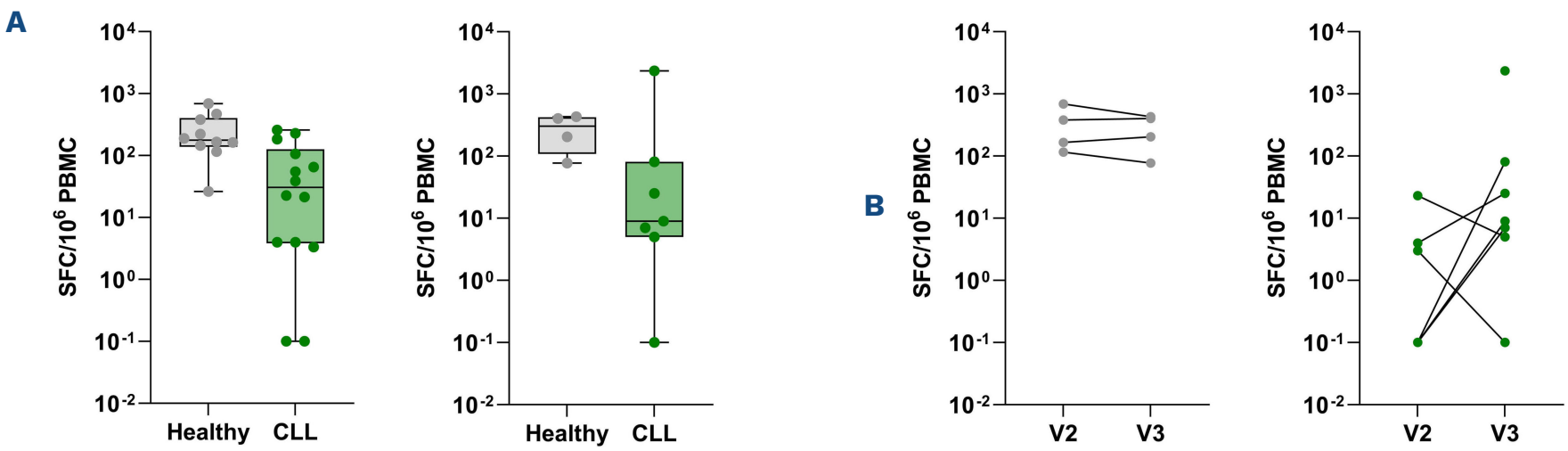

Figure 2. T-cell immune responses after COVID-19 vaccination. (A) Interferon-γ T-cell ELISpot response in chronic lymphocytic leukemia (CLL) patients and healthy controls (HC). Shown values are mean spots of duplicate wells, where background in negative control wells is subtracted from peptide-stimulated wells. The line displays the median response after second (V2) (left) and third (V3) vaccination (right). The limit of detection is 8 spot-forming cells/10^6 peripheral blood mononuclear cells. Samples were acquired 28 days after V3 in HC and at a median of 47 and 31 days (V2 and V3, respectively) in CLL patients. (B) Individual course of Interferon-γ T cell ELISpot response in HC (left) and CLL patients (right) after V2 and V3.

We found that anti-SARS CoV-2 antibodies were higher in patients who received three doses of BNT162b2 compared to two doses of BNT162b2 and a vector vaccine as booster, but that the latter vaccine combination was able to mount a serologic response in two of three previously negative patients. Yet, neutralizing serum activity was only partly detectable. In order to elicit a neutralizing serum response, a fourth dose might be beneficial by further increasing IgG levels.[10,11]

We can confirm previous data from immunocompromised patients with rheumatological disease,[7] solid organ transplantation[8] and solid malignancies[12] within our CLL cohort revealing that T-cell responses are enhanced following V3. Further indepth analyses may provide insights into their (poly-)functionality, proliferation capacity, or epigenetic profile change after (booster) vaccination despite the low response-altitude and whether the response is biased towards CD4+ or CD8+ T cells.

Interestingly, all patients who received a heterologous boost (vector vaccine) showed an increased T cell response compared to our previous analysis, while only one of three after homologous boost. This supports recently published data from randomized controlled as well as observational studies suggesting a benefit of a heterologous boost for eliciting stronger T-cell responses compared to homologous immunization.[4,13] If this offers additional protection for patients with low or absent neutralizing antibodies is yet unclear, particularly considering the low response levels with respect to quantity. Considering recent data on SARS-CoV-2-specific T cells from patients with agammaglobulinaemia[14,15] showing protection from severe disease and even in patients infected with variants

of concern,[16] we hypothesize a potential benefit of increased T-cell immunity. The impact of a fourth vaccine dose on altitude and functionality of T cells should be subject of forthcoming studies.

A limitation of this study is the small sample size. In addition, our small cohort consists of mostly male and comparably old patients. Male sex and advanced age known as relevant factors for an impaired immune response which likely affect our results, but also reflect the CLL patient population well.

In conclusion, we demonstrate an inferior T-cell response to COVID-19 vaccines in CLL patients as compared to HC, but possibly higher capacity in those patients to boost such response by V3 COVID-19. While the ideal primeboost regime is yet to determine, our data encourage to evaluate heterologous immunization by clinical trials in CLL patients.

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Contributions

SCM and PL implemented the research and design of the study. They were responsible for data assessment, coordination and conduct of the study and authored the manuscript. LM performed the T-cell vaccine response laboratory analyses and co-authored the manuscript. HG and KV performed the humoral vaccine response laboratory analyses and co-authored the manuscript. HAS, MS and MT performed blood sample processing and co-authored the manuscript. LMW, SR, CD, MMA, FK, AMF, KF, BE and MH supervised the conduct of the study, gave advice for study design and laboratory analyses and co-authored the manuscript.

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Data-sharing statement

Data may be available upon request to the corresponding author.

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