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Sibylle C. Mellinghoff, Leonie Mayer, Sandra Robrecht, Leonie M. Weskamm, Christine Dahlke, Henning Gruell, Malike Schlotz, Kanika Vanshylla, Hans-A. Schloesser, Martin Thelen, Anna-Maria Fink, Kirsten Fischer, Florian Klein, Marylyn M. Addo, Barbara Eichhorst, Michael Hallek and Petra Langerbeins

1 Faculty of Medicine and University Hospital of Cologne, Department I of Internal Medicine, Centre for Integrated Oncology Aachen Bonn Cologne Düsseldorf (CIO ABCD), University of Cologne, Cologne, Germany
2 German Centre for Infection Research (DZIF), partner site Bonn-Cologne, Cologne, Germany
3 Department of Clinical Immunology of Infectious Diseases, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
4 Division of Infectious Diseases, First Department of Medicine, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany
5 German Centre for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Riems, Hamburg, Germany
6 Institute of Virology, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany
7 Centre for Molecular Medicine Cologne, University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany

* These authors contributed equally to this work.

Corresponding author
Dr. med. Sibylle C. Mellinghoff, Department I for Internal Medicine, University Hospital of Cologne, Kerpener Str. 62, 50937 Cologne, Germany; sibylle.mellinghoff@uk-koeln.de; Tel. +49 221 478 85523/Fax +49 221 478-1435226

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Competing interests
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outside the submitted work. MH reports other from Abbvie, other from F. Hoffman-LaRoche, other from Gilead, other from Janssen-Cilag, other from Mundipharma, during the conduct of the study. PL reports grants and personal fees from Janssen-Cilag, personal fees from Abbvie, other from F. Hoffman-LaRoche, personal fees from AstraZeneca. SR reports honoraria from AstraZeneca.

The remaining authors declare no competing financial interests for this study.

**Authorship 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.

**Data sharing statement**

Data may be available upon request to the corresponding author.

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**Tables**: 1

**References**: 16

The CLL-registry is a multicenter observational study (NCT02863692).
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.\(^3\)

Blood samples of CLL registry (NCT02863692) patients were evaluated after three COVID-19 vaccinations. Six of the initially 21 patients\(^3\) were included into the analyses, 3 with homologous and 3 with heterologous vaccination schedule (mean interval between 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 IgG antibodies, determined using the Alinity ci SARS-CoV-2 IgG II Quant assay (Abbott), were detectable in 4/6 (66.7%) CLL patients after compared to 2/6 (33.3%) before booster vaccination (Figure 1A), cut-off ≥ 7.1 BAU/ml. In the one individual with detectable RBD-specific IgG after second vaccination, the third vaccination resulted in increased levels. In another individual, the third vaccination raised the IgG titer to similar levels as seen shortly after second vaccination (Figure 1B and 1C). 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 (PBMCs) were used for SARS-CoV-2 spike-specific T cell analyses (Human IFNy ELISpot\(^\text{plus}\) [ALP] kit [Mabtech]). Results are reported as spot-forming cells (SFC) per million PBMCs. 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 2nd BNT162b2 vaccination was 31 SFC (interquartile range [IQR] 4.0-96.0) (Figure 2A). The response after 2nd vaccination in the here described subgroup was significantly lower (1.7 SFC [IQR 0.0-3.8]) but increased to 8 SFC (interquartile range [IQR] 5.7-21.3) after booster vaccination. Overall, 4/6 (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 3rd vaccination. 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 boost that received a heterologous booster immunization showed an increase in T cell response. In contrast, homologous booster led to an increase in only 1/3 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 2/6 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 3rd 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.\textsuperscript{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.\textsuperscript{7,8}

We here report an increase of the humoral response in CLL patients after a 3rd COVID-19 vaccine despite B cell-depleting treatment, as reported elsewhere\textsuperscript{9}, and in addition, an increase of the cellular response in 4/6 patients.

Our data show that a 3rd immunization enhances IgG response in CLL patients, also in those that lacked detectable IgG after two vaccinations. 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 2/3 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.\textsuperscript{10,11}

We can confirm previous data from immunocompromised patients with rheumatological disease\textsuperscript{7}, solid organ transplantation\textsuperscript{8} and solid malignancies \textsuperscript{12} within our CLL cohort revealing that T cell responses are enhanced following the 3rd vaccination. Further in-depth 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 1/3 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.\textsuperscript{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\textsuperscript{14,15} showing protection from severe disease and even in patients infected with variants of concern\textsuperscript{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 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 a third COVID-19 vaccine. While the ideal prime-boost regime is yet to determine, our data encourage to evaluate heterologous immunization by clinical trials in CLL patients.
## ORCID profiles

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<tr>
<td>Sibylle Mellinghoff</td>
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</tr>
<tr>
<td>Petra Langerbeins</td>
<td>0000-0002-6654-0304</td>
</tr>
<tr>
<td>Leonie Marie Weskamm</td>
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<tr>
<td>Leonie Mayer</td>
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</tr>
<tr>
<td>Christine Dahlke</td>
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</tr>
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<td>Marylyn M. Addo</td>
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<td>Martin Thelen</td>
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<td>Anna Maria Fink</td>
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</tr>
<tr>
<td>Henning Gruell</td>
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<td>Kanika Vanshylla</td>
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References


**Table 1.** Patient Characteristics and outcomes.

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**Healthy Control**

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*S-specific T cells [SFC/10^6 PBMC] **RBD-specific IgG [BAU/ml] ***Neutralization activity [Serum ID_{50} Wu01 Pseudovirus]

Figure legends

Figure 1. Humoral immune responses after COVID-19 vaccination. (A) Antibody response rate in CLL patients after 2nd and after 3rd vaccination. (B) SARS-CoV-2 RBD-specific IgG in CLL patients after 2nd and 3rd vaccination (median 10.05 BAU/ml, range 0.1- 10998.6) measured by CMIA. (C) Individual course of IgG antibodies in CLL patients after 2nd and 3rd vaccination. (D) Serum neutralizing activity (50% inhibitory serum dilution) determined in a pseudovirus neutralizing assay against the Wu-01 pseudovirus strain. Bars indicating geometric mean ID50 with 95% confidence intervals. Dashed line indicates limit of detection (LOD, 10). Samples with no detectable neutralization (ID50 <10) were plotted with an ID50 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 CLL patients and 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 2nd (left) and 3rd vaccination (right). The limit of detection is 8 SFC/10^6 PBMCs. Samples were acquired 28 days after 3rd vaccination in HC and at a median of 47 and 31 days (2nd and 3rd, respectively) in CLL patients. (B) Individual course of interferon-γ T cell ELISpot response in HC (left) and CLL patients (right) after 2nd and 3rd vaccination.

Abbreviations: CLL – Chronic lymphocytic leukemia, HC - Healthy Control.
Figure 1. Humoral response to COVID-19 vaccines after 2nd and 3rd vaccination.
Figure 2. T cell response to COVID-19 vaccines after 2nd and 3rd vaccination.