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T cell immune responses following vaccination with mRNA BNT162b2 against SARS-CoV-2 in patients with chronic lymphocytic leukemia: results from a prospective open-label clinical trial

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Author contributions: LH, AÖ, MB and HGL contributed to conceptualization, funding acquisition and discussion of data. DW, PC, YG, AC, JL, ORB and MA contributed to sample processing throughout the COVAXID clinical trial. DW performed experiments and analyzed data. LB, LH and AÖ recruited CLL study participants, conducted investigation through recruitment of the study participants and conducted management of participants during the trial and analysed data. SA was the PI of the COVAXID clinical trial, contributed to conceptualization, funding acquisition and discussion of data. JL recruited CLL study participants including those off BTK inhibitor-treatment and discussed data. LB, DW, AÖ, LH, MB and HGL 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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The COVID-19 pandemic has seriously affected patients with CLL (1). Fatalities exceeding 30% has been reported among hospitalized patients in international surveys (2, 3), and in consecutively identified CLL patients (4). Additionally, many patients with CLL do not achieve seroconversion upon mRNA vaccination (5, 6). We recently confirmed those observations in the course of a prospective clinical trial involving the BNT1622b2 mRNA vaccine (7). The study included five equally sized cohorts of patients with different types of immunocompromised disorders (total n=449) and 90 healthy controls. Sixty-three percent of patients within the specific CLL cohort (n=90) seroconverted after two vaccine doses. The lowest seroconversion rate was found in patients on ibrutinib followed by those who had stopped BTK inhibitor (BTKi)-therapy (7). Even though T cell responses may occur in most patients with CLL following COVID-19 infection (4), there is still limited data on T cell immunity following vaccination of many immuno-compromised patient groups including CLL. In the first report on hematological malignancies, 9/18 patients developed SARS-CoV-2-specific T cell reactivity (8). Similar results were seen in a large patient group applying a whole blood IFN-γ release assay (9).

Early study (10) of T cell responses identified IFN-γ as a key cytokine produced by spike-specific CD4+ and CD8+ T cell in BNT162b1 mRNA vaccinated individuals. We report here on T cell immunity in patients with CLL from our above mentioned prospective clinical trial (7) using IFN-γ ELISpot, a validated quantitative assay to measure T cell responses against SARS-CoV-2-specific peptides (4, 11).

Inclusion criteria and monitoring of our vaccine clinical trial has been described earlier (7). Patients with a previous history or sign of COVID-19, or that tested positive for SARS-CoV-2 or had spike protein-specific antibodies at baseline were excluded. No patient with CLL developed break-through infection during the study or early follow-up. Fifty-two predefined patients from the CLL cohort (n=90) were subject to repeated analysis of T cell immunity against SARS-CoV-2-specific peptides. Baseline characteristics of the patients and controls are shown in Table 1. Patients included groups of previously untreated (n=14), ongoing ibrutinib therapy (n=14), had stopped ibrutinib ≥2 months ago due to remission as part of another study addressing intermittent ibrutinib treatment (n=10), or had received CD20 mAb-containing chemoimmunotherapy 6-30 months ago (of which 11 received their last dose >12 months ago) (n=14). Cellular and humoral immunity was measured at day (d) 0 (baseline), d10 (10 days after second vaccine dose), d21 and d35 (2 weeks after the second vaccine dose).
Seroconversion and antibody titres were analysed as reported (7) with d35 data available in 48 of the 52 pre-defined patients who were analysed for T cell immunity. Additionally, 41 of 90 healthy controls in the trial (7) were pre-selected for T cell analysis (Table 1).

The ELISpot assay was applied as previously described (11), using plates and reagents from a human IFN-γ ELISpot kit (3420-2APT-2, Mabtech). Briefly, 2.5 x 10^5 PBCMs/well were seeded and supplemented with 0.15 µg/ml of co-stimulatory anti-CD28/CD49d (347690, BD Biosciences). Cells were stimulated for 20 h with a peptide pool covering the SARS-CoV-2 spike glycoprotein (0.5 µg/ml, LB01792; Peptides&Elephants) and equivalent DMSO in unstimulated wells. Spot-forming units (SFUs) were counted using the IRIS (Mabtech) automated reader system. Data points are presented as background corrected SFU/10^6 cells, calculated by subtracting the mean value of the corresponding duplicate unstimulated wells from the mean value of duplicate spike stimulated wells. Negative values after background correction are set to 1. The threshold for positive response corresponds to the average SFU/10^6 cells of unstimulated wells + 2 standard deviations (30 SFU/10^6 cells). Data were excluded when unstimulated wells had >100 SFU/10^6 cells.

All analyses were pre-specified as per protocol. Data is summarized using descriptive statistics such as counts, percentages, medians and range. Categorical variables are presented as cross-tabulations and distributional differences were tested using the Chi-squared test. Significance between time points with missing values was assessed using Kruskal-Wallis test with Dunn’s post-test. Correlation analysis was done using non-parametric Spearman rank correlation. P-values <0.05 were considered significant. Graphs and associated statistical tests were performed in Prism v.9 (GraphPad Software Inc.).

Seroconversion rates were in line with our full clinical trial report (7) and are summarized in Table 1 and Fig 1A. Seroconversion occurred in 29/48 patients (60%) (4/52 patients had missing serology data at d35) compared to 41/41 (100%) of controls. Time kinetics of seroconversion is shown in Fig 1A. Only 4% and 18% of patients had seroconverted at d10 and d21, respectively, followed by 60% at d35. Patients’ and controls’ responses showed similar kinetics. There was no significant increase in SARS-CoV-2-specific IgG after vaccination on d10 in either group. At d21 and at d35, both groups had a significant response compared to baseline (CLL: d0 vs d21 p<0.05), d0 vs d35 p<0.0001; controls: d0 vs d21 p<0.0001, d0 vs d35 p<0.0001).

Subgroup analysis revealed that seroconversion occurred in 11/13 (85%) of previously untreated patients, 11/12 (92%) of previously CD20 mAb-treated patients, 6/10 (60%) of
previously ibrutinib-treated and 2/14 (14%) of patients with ongoing ibrutinib therapy. The difference between patients on or off ibrutinib was significant (p=0.02). The antibody titer (U/mL; median, range) at d35 in each subgroup as above was 81.6 (0.4-3,320), 42.4 (0.4-1,343), 35.5 (0.4-559) and 0.4 (0.4-170) respectively. This compared to a median antibody titer of 2,696 U/ml (range 766-14,269) in controls (Table 1).

Longitudinal assessment of T cell immunity against SARS-CoV-2 spike peptides (ELISpot) is shown in Fig 1B and summarized in Table 1. At d35, 15/52 patients (29%) had a specific T cell response (p<0.05 vs baseline, Fig 1B) compared to 24/41 (59%) in controls (p<0.01). Pre-existing spike-cross-reactive T cells (12) was observed at baseline in 5/50 patients; 4 showed no vaccine response and 1 patient showed a marginal increase in T cell response (mean spot count from 44 to 70 SFU/10^6 PBMC at d35). A positive T cell response was observed in 9/50 tested patients (18%) at d10 and in 6/51 (12%) at d21.

CLL subgroup results are shown in Fig 1C and Table 1. IFN-γ positivity was observed in 7/10 patients at d35 who were off ibrutinib, whereas only 2/14 patients on ibrutinib developed T cell immunity (p<0.01). The corresponding numbers were 4/14 among previously untreated patients and 2/14 if previously treated with CD20 mAbs (p<0.05 and p<0.01, respectively, vs patients off ibrutinib).

Finally, we analysed correlation between seroconversion and T cell response (Fig 1D). A weak but significant correlation was observed (r = 0.2861, p = 0.049). Fifteen patients (29%) were double-negative; i.e., neither mounted a T cell response nor seroconverted, whereas 9 (18%) came out positive in both assays. Twenty patients (39%) were positive in serology only and only 3 patients (2 in the off ibrutinib group) had an IFN-γ response in the absence of seroconversion. Most double-negative patients (11/15) were found among patients on ibrutinib. Double-positive patients were most frequent in those off ibrutinib (4/9). Of the 20 seroconverted patients with no T cell response, one patient was found in the ongoing ibrutinib and one in the previously ibrutinib treated group, while 8 were previously untreated and 10 were previously treated with CD20 mAb.

Following natural COVID-19 infection, durable immunity including both antibodies and T cells seem to occur in most healthy individuals (13) as well as in patients with CLL (4). Most healthy individuals mount T cell responses following mRNA vaccination (14). This was reported also in patients with solid tumors (15). Lower numbers were recently reported in patients with hematological malignancies (8, 9). The present study shows that, compared to healthy controls, half as many of patients with CLL developed IFN-γ T cell response (28% vs
59%) after two doses of mRNA vaccine. A limitation of the present (11) T cell assay is capturing of only IFN-\(\gamma\) positive cells, e.g., missing-out on other cytokine-secreting antigen-specific T cells. Despite this, we were able to capture both temporal and group dynamic changes of the SARS-CoV-2 spike-specific T cell response, and were able to make comparison of patients with CLL with healthy controls. CLL subgroup results were driven by patients who were off ibrutinib (7/10 responded) whereas other CLL sub-groups had few T cell responders. However, the data must be viewed with caution due to the open-label trial design and the small numbers within each subset. Thus, our subgroup analysis should be confirmed in extended studies. Even though our healthy controls were younger (median age 52 years) age did not impact on their T cell response (data not shown). Double-negativity was found in most patients on ibrutinib who remain of major concern, suggesting that temporary cessation of BTKi may be explored onwards in future studies. Of note, Ehmsen et al. (9) found T cell responses in 26% of seronegative hematology patients whereas we found it only in 3/52 patients with CLL (6%). A third dose is currently explored in CLL and its effect on T cell immunity, even though its additional effect on T cells was limited in solid tumors (15). CLL remain as a group of special concern in the ongoing pandemic.
References


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Table 1. Baseline characteristics and immunological results 2 weeks after the second dose (d35) of the mRNA BNT162b2 vaccine in relation to CLL patient subgroup and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Entire cohort n=52</th>
<th>Indolent untreated n=14</th>
<th>Previous CD20 mAb n=14</th>
<th>Previous ibrutinib n=10</th>
<th>Ongoing ibrutinib n=14</th>
<th>Healthy controls n=41</th>
</tr>
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<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>median (range)</td>
<td>70 (23 – 86)</td>
<td>71 (49 – 82)</td>
<td>71 (56 – 84)</td>
<td>71 (54 – 86)</td>
<td>70 (23 – 84)</td>
<td>52 (25–79)</td>
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<tr>
<td><strong>Male sex</strong></td>
<td></td>
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<tr>
<td>n (% )</td>
<td>36 (69%)</td>
<td>6 (43%)</td>
<td>13 (93%)</td>
<td>6 (60%)</td>
<td>11 (79%)</td>
<td>19 (46%)</td>
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<tr>
<td><strong>Seroconverted d35</strong></td>
<td></td>
<td></td>
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<tr>
<td>n (%)</td>
<td>29/48 (60%)</td>
<td>11/13 (85%)</td>
<td>11/12 (92%)</td>
<td>5/9 (56%)</td>
<td>2/14 (14%)</td>
<td>41/41 (100%)</td>
</tr>
<tr>
<td><strong>Ab titres d35, U/ml</strong></td>
<td></td>
<td></td>
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<tr>
<td>median (range)</td>
<td>24.6 (0.4 – 3320)</td>
<td>81.6 (0.4 – 3320)</td>
<td>42.4 (0.4 – 1343)</td>
<td>35.5 (0.4 – 559)</td>
<td>0.4 (0.4 – 170)</td>
<td>2696 (766–14269)</td>
</tr>
<tr>
<td><strong>ELISpot d35, SFU/10^6 PBMC</strong></td>
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<tr>
<td>median (range)</td>
<td>10 (1 – 1096)</td>
<td>6 (1 – 526)</td>
<td>10 (1 – 490)</td>
<td>53 (2 – 1096)</td>
<td>5 (2 – 70)</td>
<td>48 (1-1526)</td>
</tr>
<tr>
<td><strong>Positive T cell response</strong></td>
<td></td>
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<tr>
<td>n (%)</td>
<td>15/52 (29%)</td>
<td>4/14 (29%)</td>
<td>2/14 (14%)</td>
<td>7/10 (70%)</td>
<td>2/14 (14%)</td>
<td>24/41 (59%)</td>
</tr>
</tbody>
</table>

*Ab baseline = 0.4 U/ml; Seropositive >0.8 U/ml.

*a n = 48 (with d35 data)

Abbreviations: Ab; antibodies; ELISpot; enzyme-linked immunospot; SFU; spot forming units; PBMC; peripheral blood mononuclear cells.
Figure legend

**Fig. 1: Humoral and cellular immune response in CLL patients.** Longitudinal assessment (day 0, 10, 21, 35 post-vaccination) of SARS-CoV-2-specific IgG (A) and IFN-γ T cells (B) after spike glycoprotein stimulation, with summarized number and frequency of patients tested positive. Subgroup analysis of CLL patients at day 35 (C) and correlation day 35 (D). The dashed line indicates positive threshold for SARS-CoV-2-specific IgG and IFN-γ SFU/10⁶ cells, 0.8 U/ml and 30 SFU/10⁶ cells respectively. The dotted line represents the lower limit of detection of both assays. Each dot represents one patient. Rₚ = Spearman r value. Kruskal-Wallis test with Dunn’s correction for multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.