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# Hybrid immunity in immunocompromised patients with CLL after SARS-CoV-2 infection followed by booster mRNA vaccination

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**Abstract:**

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# Hybrid immunity in immunocompromised patients with CLL after SARS-CoV-2 infection followed by booster mRNA vaccination

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Patients with chronic lymphocytic leukemia (CLL) are immunocompromised[1]. They are at high-risk for developing severe COVID-19[2-4] and mount suboptimal immunity after mRNA vaccination[5, 6], but with slightly conflicting results regarding cellular immunity[7, 8]. In a "hybrid immunity" setting, immune protection is influenced by prior infection status and vaccination. Here, we studied humoral and cellular hybrid immunity in a CLL cohort of twenty-nine patients with a history of COVID-19[2] and three consecutive mRNA vaccinations against SARS-CoV-2 (two patients received two doses).

The study was approved by Swedish Ethical Review Authority (www.etikprovningsmyndigheten.se). Written informed consent was obtained before sampling. Serum and saliva antibodies were measured pre-vaccination, after dose two, and before and after dose three vaccinations. SARS-CoV-2 IgG antibodies in serum were analyzed using Roche Elecsys qualitative anti-SARS-CoV-2 and quantitative anti-SARS-CoV-2 immunoassays as described[2, 5]. Saliva was collected using a self-sampling technique, and SARS-CoV-2 IgG antibodies were analyzed as described[9-11]. T cell responses were analyzed by activation-induced marker (AIM) assay as described[12] before vaccination and before and after dose three. We also applied the IFN-γ ELISpot as described[2, 7]. Experimental methods are described in Supplemental Methods.

Twenty-nine patients from a previous COVID-19 study were included[2]. Patient characteristics and mRNA vaccinations are summarized in Table 1 and Supplemental Table 1. Twenty-one patients were untreated, six had received CD20 mAb-containing treatment >12 months ago, and two received BTKi therapy. The variability in time since recovery from COVID-19 and between vaccinations and tests are depicted in Table 1. Break-through infections were not observed during the study (until Dec 21, 2021). Immune responses are shown in Figure 1 (entire cohort) and Supplemental Figure S1 (patients without missing data points). Serology results are shown in Figure 1A. Ninety-three percent of patients (26/28) were seropositive before the first vaccination with titer levels available in 25 patients (Figure 1A). The median spike-specific antibody titer was 47 U/ml (range <0.8–911), including three seronegative patients. After two doses, 96% (24/25) were seropositive and the median antibody titer had increased to 17,208 U/ml (range <0.8–>25,000, IQR 2,793->25,000) ($p<0.0001$). This titer fell to a median of 6,825 U/ml (range <0.8–>25,000, IQR 2,532->25,000) prior to dose three and then increased to a median of 24,956 U/ml following dose three (range <0.8–>25,000 U/ml, IQR 4,219->25,000) ($p<0.0001$) (upper detection limit 25,000 U/ml). Nucleocapsid-specific antibody levels tended to decline over time (Figure 1A).

Next, we analyzed salivary IgG and 95% of patients (19/20) had spike-specific IgG prior to dose three (Figure 1B), with a moderate correlation between spike antibodies in serum and saliva ($r=0.4622$ $p<0.0006$) (Supplemental Figure S2A). Salivary spike IgG levels appeared with slower kinetics and increased before dose three ($p<0.05$) (Figure 1B). The average level before and after dose three was comparable to naive healthy controls after two vaccine doses[10]. Nucleocapsid reactivities were stable with a transient rise before dose three ($p<0.05$) (Figure 1B).

Thereafter, we analyzed T cell responses against wildtype Wu-Hu1 and Omicron (BA.1) as described[12]. Thirteen patients were included, with memory responses shown in Figure 1C. After dose three, the spike-specific-CD4+ T cells ($p<0.05$) (Fig 1D) and CD8+ T cells ($p<0.01$) increased (Figure 1E) with a similar magnitude for Wu-Hu1 (wildtype) and Omicron (BA.1).

IFN-γ ELISpot analysis showed no significant differences following dose three (Figure 1F), although spike-specific responses tended to increase while non-spike-specific responses (M+E+N) declined over time.

Additionally, we also made paired analyses of results in patients without missing data points (Supplemental Figure S1). Almost identical and statistically significant changes over time were observed in this group compared to the entire cohort for spike antibodies in serum (n=19, Figure S1A) and saliva (n=14, Figure S1B) and for CD8+ T cells (n=7, Figure S1D). The change in CD4+ T cells (n=7, Figure S1C) did not any longer reach statistical significance. In contrast, the change in nucleocapsid antibody levels was more pronounced than in the entire cohort, decreasing in serum (n=13, Figure S1A) and increasing in saliva (n=14, Figure S1B).

Patients with CLL have shown low anti-spike titers following mRNA-vaccination against SARS-CoV-2[5, 6] even after three doses[13]. Primary COVID-19 infection resulted in higher titers and T cell responses[2], although, as shown here, at low levels at the time of first vaccination. The present results mimic those observed of hybrid immunity in otherwise healthy individuals that a combined effect of infection and vaccination results in robust humoral and cellular anti-SARS-CoV-2 immunity[14].

To the best of our knowledge, the present study is the first to report on hybrid immunity in patients with CLL. The median serology titers of 17,208-24,956 U/ml after two and three doses were not affected by the analytical range of the assay even though several patients had serology titers above the upper level 25.000 U/ml (Figure 1A). Titers are markedly higher

than the median of <100 U/ml that were found after two vaccine doses in non-COVID patients with CLL who participated in an earlier prospective vaccine trial[5]. This includes abundant local immunity in saliva[5]. The plateau at dose three in the present study is in line with four vaccine doses in healthy individuals[15]. Saliva antibodies followed a slower time kinetics compared to serum, not being significantly increased until pre-dose three. Whether this is due to rebound infection limited to the oral cavity[16] is unknown but partly supported by the increase in nucleocapsid-specific IgG. Robust spike-specific CD8+ T cell responses occurred after vaccine dose three, in line with a report on patients with multiple sclerosis receiving three vaccine doses[17]. T cell immunity was also assessed against Omicron (BA.1), with higher CD8+ T cell magnitudes after three doses in the CLL cohort than in healthy non-COVID-19 individuals after two mRNA vaccine doses[12].

There are limitations with the present study. The cohort is limited, most patients had early-stage CLL, and few had ongoing therapy, which may affect immune responses favorably. The variation in time between COVID-19 and the start of vaccination and between vaccinations may affect the magnitude of the immune responses. Neutralizing antibodies were not measured even though we found a strong correlation between serum and saliva IgG levels with neutralization in the same patients earlier[2] and confirmed by others[18]. Finally, there was no control group tested in parallel with this real-world cohort and the groups studied (healthy donors and CLL) were not COVID-19 convalescents and received only two vaccine doses at the time of reporting[5, 7, 10, 12].

Hybrid immunity was recently reported to confer long-lasting protection from severe disease in healthy persons[19-21] even though not preventive against Omicron[22]. The serial measurement of systemic B and T cell responses were spike-restricted. Nucleocapsid-directed immune responses were relatively stable over time, albeit with a slow decline, 9-20 months after COVID-19. Also, nucleocapsid antibody levels were stable or showed a slight increase in saliva, which may serve as a first-level defense barrier against re-infection by ancestral SARS-CoV-2 strains[16, 23]. In conclusion, we demonstrate robust hybrid immunity in serum, saliva and the T cell compartment in patients with CLL who received three doses of mRNA vaccine following COVID-19 infection. The results are encouraging in the context of immunocompromised patients who have recovered after COVID-19 and need continuous protection against new SARS-CoV-2 variants-of-concern. To obtain protection, patients who remain seronegative shall be offered available anti-SARS-CoV-2 preventive therapies.

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**Authorship contributions**

LB, LH, AÖ, GB, MSC, HGL, and MB contributed to the conceptualization, funding acquisition, and discussion of data. YG, KH and SM, and DW 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. LB, DW, AÖ, LH, 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.

**Disclosure of conflicts of interest**

The authors declare no competing financial interests. MB is a consultant for Oxford Immunotech. AS is a consultant for Gritstone Bio, Flow Pharma, Arcturus Therapeutics, ImmunoScape, CellCarta, Avalia, Moderna, Fortress, and Repertoire. LJI has filed for patent protection for various aspects of T cell epitope and vaccine design work.

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Table 1. Clinical characteristics at start of mRNA vaccination against SARS-CoV-2 as well as timepoints of vaccination and tests in patients with CLL (n=29) with a prior history of COVID-19 infection

| | |
|---|---|
| Median age, years (range) | 65 (47 – 83) |
| Male/Female | 20/9 |
| **CLL treatment status** | |
| Never treated | 72% (21/29) |
| Previously treated[a] | 21% (6/29) |
| Time (mo) since last treatment | 45.5 (15 – 70) |
| Ongoing therapy[b] | 7% (2/29) |
| Ongoing Ig supplement | 10% (3/29) |
| **CLL stage (Rai)** | |
| 0 | 83% (24/29) |
| I-II | 17% (5/29) |
| III-IV | 0% (0/29) |
| **CLL remission status (iwCLL)** | |
| SD | 0% (0/29) |
| PD | 0% (0/29) |
| PR/CR | 34% (10/29) |
| Not applicable (never treated, early stage) | 62% (18/29) |
| Not applicable (never treated, progressive disease) | 3% (1/29) |
| **Time (mo) since Covid-19 diagnosis to vaccination, median (range)** | |
| Dose 1 (n=29) | 5.75 (1.75-13.75) |
| Interquartile range | 4.00-11.25 |
| Dose 3 (n=27)[c] | 12 (7.75-19.75) |
| Interquartile range | 9.75-17.75 |
| **Time (mo) since pre-test to vaccination, median (range)** | |
| Dose 1 (n=28)[c] | 0.25 (0-4.25) |
| Interquartile range | 0-1.00 |

| | |
|---|---|
| Dose 3 (n=25)[c] | 1.25 (0.25-2.75) |
| Interquartile range | 1.00 -1.75 |
| **Time (mo) since vaccination to test, median (range)** | |
| Dose 2 (n=25)[c] | 0.75 (0.25-2.5) |
| Interquartile range | 0.51-1.00 |
| Dose 3 (n=27)[c] | 0.75 (0.5-1.5) |
| Interquartile range | 0.75-1.00 |
| **Time (mo) between vaccine doses, median (range)** | |
| Dose 1-2 (n=29) | 1.5 (0.75-2.0) |
| Interquartile range | 1.37-1.50 |
| Dose 2-3 (n=27) | 5 (2.75-8.25) |
| Interquartile range | 4.25-5.75 |
| **Type of vaccine**[d] | |
| Dose 1 (n=29) C=25, S=4 | |
| Dose 2 (n=29) C=25, S=4 | |
| Dose 3 (n=27) C=25, S=2 | |
| **Covid management**[e] | |
| Hospital admission | 66% (19/29) |
| ICU admission | 7% (2/29) |
| Supplemental oxygen | 48% (14/29) |
| Corticosteroids | 31% (9/29) |
| Antiviral therapy (Remdesivir) | 14% (4/29) |
| Anticoagulation | 59% (17/29) |
| IvIg | 3% (1/29) |
| Convalescent plasma | 0% (0/29) |
| BTKi | 3% (1/29) |
| Tocilizumab | 0% (0/29) |
| Hydroxychloroquine | 3% (1/29) |

[a] With no current treatment. All with anti-CD20 mAb containing immunochemotherapy (ICT) (BR/FCR) and all >12 months prior to vaccination

[b] Both with BTKi (ibrutinib). One was previously treated with ICT >12 months ago and stopped ibrutinib therapy shortly after the 2nd vaccine dose.

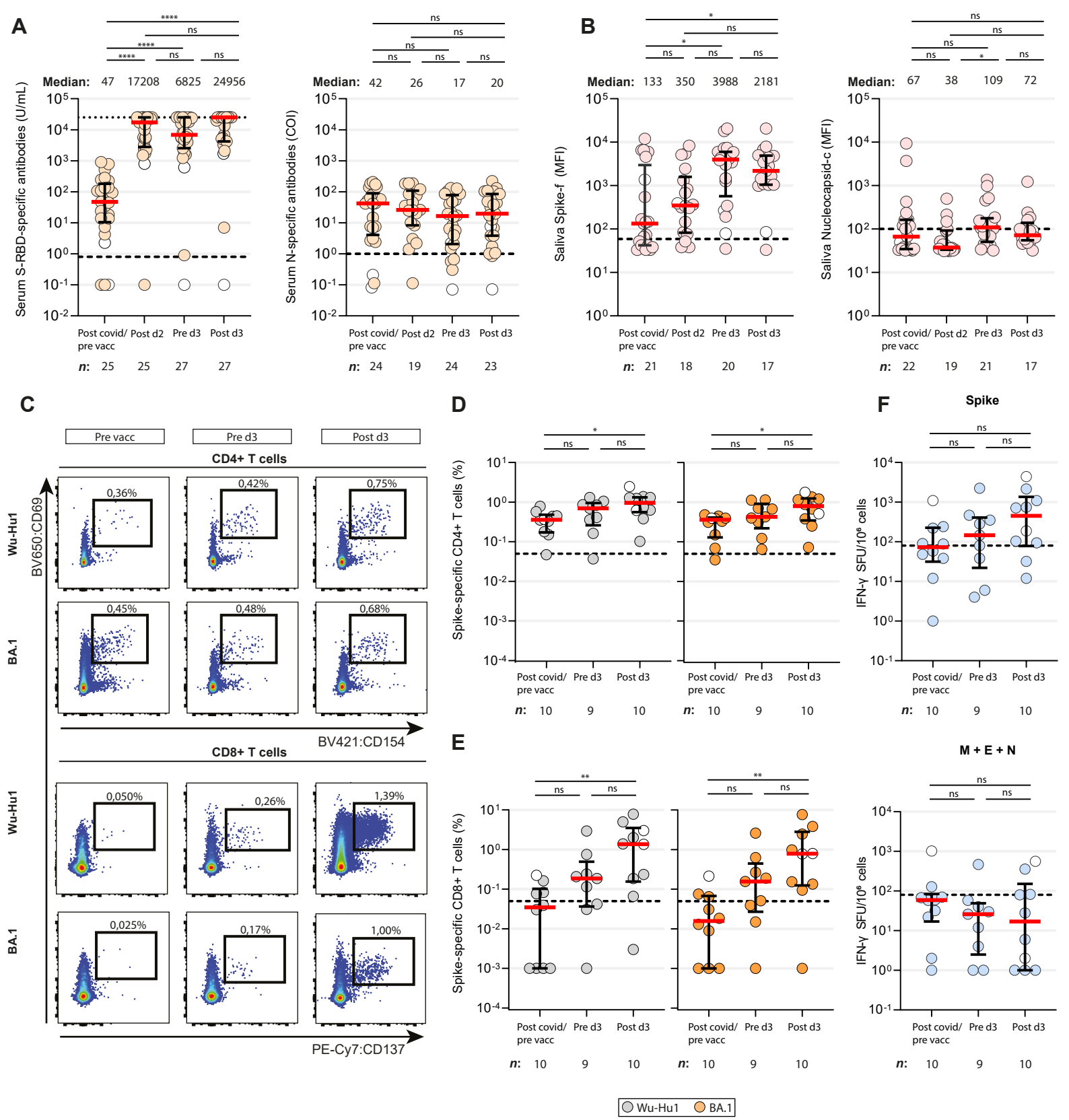
[c] Number of patients at each time point is shown in Fig 1 and Supplemental Fig S1.

[d] C= Comirnaty (BNT162b2, Pfizer BioNTech), S= Spikevax (mRNA-1273, Moderna). Two patients did not receive dose 3

[e] March 2020-March 2021

## Figure legends

**Figure 1. Time kinetics of humoral and cellular responses against spike and non-spike epitopes of SARS-CoV-2 in CLL patients after COVID-19 infection followed by three mRNA vaccination doses.** (A) Serum and (B) salivary levels of S (spike)-receptor-binding domain (RBD) and N (nucleocapsid)-specific antibody responses in patients post-COVID-19 and pre-vaccination, post-vaccination (dose 2), pre-vaccination (dose 3) and post-vaccination (dose 3) with indicated median values. (C) Spike-specific CD4+ (CD69+CD154+) and CD8+ (CD69+CD137+) T cells were detected by flow cytometry (AIM assay). Spike-specific CD4+ (D) and CD8+ (E) T cell response against Wu-Hu1 (wildtype) and Omicron (BA.1) post-COVID-19 and pre-vaccination, pre-vaccination (dose 3) and post-vaccination (dose 3). (F) ELISpot IFN- γ -specific T-cell responses to spike and membrane, envelope and nucleocapsid (M+E+N) peptide pools post-COVID-19 or pre-vaccination, pre-vaccination (dose 3) and post-vaccination (dose 3). White dots represent patients on BTKi treatment in the respective analysis. Number (*n)* of patients tested at each time point is indicated below graph. Assay upper limit of detection of 25,000 U/mL is shown as dotted line (A). Dashed line represents positive threshold for each assay: (A) 0.8 U/ml and 1 cut-off index (COI) respectively, (B) median fluorescence intensity (MFI) of 59 and 100 for S and N respectively, (D) and (E) 0,05% and (F) 80 spot-forming units (SFU)/$10^6$ cells. Error bars represent the median (red line) and interquartile range. Kruskal-Wallis test with Dunn's multiple comparison correction was used, *P<0.05, **P< 0.01, ****P<0.0001.


A
Median: 47 17208 6825 24956
Serum S-RBD-specific antibodies (U/mL)
Median: 42 26 17 20
Serum N-specific antibodies (COI)
Post covid/ pre vacc
Post d2
Pre d3
Post d3
n: 25 25 27 27
n: 24 19 24 23
B
Median: 133 350 3988 2181
Saliva Spike-f (MFI)
Median: 67 38 109 72
Saliva Nucleocapsid-c (MFI)
n: 21 18 20 17
n: 22 19 21 17
C
Pre vacc
Pre d3
Post d3
CD4+ T cells
Wu-Hu1
BA.1
BV650:CD69
BV421:CD154
0,36%
0,42%
0,75%
0,45%
0,48%
0,68%
CD8+ T cells
0,050%
0,26%
1,39%
0,025%
0,17%
1,00%
PE-Cy7:CD137
D
Spike-specific CD4+ T cells (%)
E
Spike-specific CD8+ T cells (%)
F
Spike
M + E + N
IFN-γ SFU/10⁶ cells
Wu-Hu1
BA.1