

# Safety and immunogenicity of the BNT162b2 mRNA Covid-19 vaccine in patients with chronic lymphocytic leukemia: a prospective study

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## Abstract

**Introduction:** Immunization of patients with chronic lymphocytic leukemia (CLL) with vaccines against several infectious diseases has proven insufficient. Data on seroconversion of patients with CLL after vaccination against severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) are still young, but accumulating evidence shows low seroconversion rates.

**Methods:** We conducted a prospective, noninterventional study evaluating the safety and immunogenicity of two doses of the BNT162b2 mRNA Covid-19 vaccine, administered 21 days apart in consecutive adult patients with CLL. Patients vaccinated with other vaccines against SARS-CoV-2, with a history of confirmed Coronavirus Disease 19 (COVID-19), with known human immunodeficiency virus infection, or with an inability to provide written informed consent were excluded. Sera were tested before the first and after the second dose of the vaccine for anti-SARS-CoV-2 receptor binding domain (RBD) spike protein IgG (anti-RBD), using the Abbott SARS-CoV-2 IgG II Quant assay (Abbott Laboratories, Abbott Park, IL, USA), with a cutoff value for seroconversion at 50 AU/ml.

**Results:** Sixty-one patients (28 males/33 females) with CLL, with a median age of 61 years, were included in the study. The majority of the patients (82.0%) were lower (0–2) stage per the Rai staging system. The seroconversion rate at 14 days after the second dose was 45% and was correlated with Rai stage (0–2 versus 3–4; 51.0% versus 18.3%,  $p=0.047$ ), the treatment status (treatment naïve, previously treated, or actively treated patients; 63.0% versus 40.0% versus 26.1%, respectively,  $p=0.031$ ), the number of previous treatment lines (0–2 versus >2; 55.3% versus 8.3%,  $p=0.004$ ), and the platelet count of the patients (over or under  $100 \times 10^9/L$ ; 52.9% versus 10.0%,  $p=0.015$ ). Moreover, there was a positive linear relationship between the antibody titers and the gamma-globulin levels ( $r=0.182$ ,  $p=0.046$ ) and platelet count ( $r=0.277$ ,  $p=0.002$ ). Finally, patients actively treated with venetoclax had higher antibody titers than those treated with ibrutinib (15.8 AU/ml versus 0.0 AU/ml,  $p=0.047$ ). No safety issues were identified while the emergence of adverse events was not correlated with immunogenicity.

**Discussion:** This study confirms results from previous studies on the low seroconversion rates in patients with CLL vaccinated with the BNT162b2 mRNA Covid-19 vaccine and on the detrimental effect of advanced disease and multiple treatment lines on seroconversion, while it is suggested that treatment with venetoclax may offer a chance for higher antibody titers, suggesting a treatment strategy change during the pandemic provided that this result is confirmed by larger studies specifically designed to address this issue.

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## Introduction

In the era of the Coronavirus Disease 19 (COVID-19) pandemic, the need to achieve and maintain immunization against severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) is of paramount importance, given the increased risk for serious disease, complications, and death that has been observed among people with underlying medical conditions.<sup>1-4</sup>

Vaccination has proven to be an effective way to accomplish prevention of symptomatic infection and reduction of death rate in the general population because randomized trials reported an efficacy of 94% to 95% for the available mRNA vaccines.<sup>5,6</sup> However, data concerning special patient groups are still young as the latter are excluded from vaccine trials.<sup>5,6</sup>

Patients with chronic lymphocytic leukemia (CLL) constitute one such special group with inherent and treatment-related characteristics that may affect vaccine efficacy. Patients with CLL have inherent immune defects in humoral and cell-mediated immunity that are related to the primary disease process, including hypogammaglobulinemia, abnormalities in T-cell subsets, as well as defects in complement activity and neutrophil/monocyte function, while therapy-related immunosuppression has a further impact on immune function.<sup>7</sup> As a result, response to vaccines is frequently inadequate. According to published data, patients with CLL have suboptimal immune response to the pneumococcal polysaccharide antigen, the tetanus toxoid antigen, the *H. influenzae* type b vaccine, and the varicella-zoster virus vaccine. This is especially true for patients with advanced disease and hypogammaglobulinemia and those actively treated especially with anti-CD20 antibodies and Bruton tyrosine kinase inhibitors (BTKi).<sup>8-15</sup>

Recent studies on COVID-19 and CLL<sup>3,16</sup> have reported that, regardless of disease phase or treatment status, these patients are at high risk of poor outcome, due to immunosuppression and advanced age, because CLL is mostly a disorder

of the elderly, with a median age at diagnosis of approximately 72 years.<sup>17</sup> Moreover, they suffer more frequently from persistent viremia,<sup>18</sup> and they act as a reservoir for the emergence of new mutations of the virus, thus rendering the need for effective immunization of paramount importance.

Here, we report the results from a prospective, noninterventional study evaluating the safety and immunogenicity of the BNT162b2 mRNA Covid-19 vaccine in adult patients with CLL.

## Methods

### Patients

Adult patients with CLL treated in three tertiary hospitals in Athens, Greece, were informed about the study and participated after providing a written informed consent. Patients with CLL who were willing to be vaccinated against SARS-CoV-2 according to the national vaccination program were preselected to be included in the study, but only patients vaccinated with the BNT162b2 mRNA Covid-19 vaccine were eventually included in the study. Further exclusion criteria included history of confirmed COVID-19, known human immunodeficiency virus infection, and inability to provide written informed consent. All CLL patients treated in the hematology departments were approached by the treating physicians and enrolled in a consecutive manner. The study started on 25 January 2021, and its duration was 6 months. At baseline, the epidemiological, clinical, and laboratory characteristics of the patients as well as treatment data were recorded as follows. Age and disease stage at the time of vaccination, disease duration, complete blood count parameters (hemoglobin level, lymphocyte, neutrophil, monocyte, and platelet count), and gamma-globulin levels were recorded and analyzed. Moreover, data on the treatment of the patients (treatment lines; previous treatment with anti-CD20 antibodies, fludarabine, or ibrutinib; active treatment; and treatment regimen at the time of vaccination) were

also collected and analyzed. Patients were divided into three groups based on treatment data, that is, treatment naïve, previously treated, and actively treated patients. Adverse reactions to previous vaccinations were also recorded.

### Vaccination

Patients were vaccinated with two 30 µg doses of the BNT162b2 mRNA Covid-19 vaccine administered intramuscularly in the deltoid muscle 21 days apart, according to the national program for vaccination against SARS-CoV-2.

### Study procedures

The study was designed to assess immunogenicity at baseline (i.e. within 5 days before the first dose of the vaccine) due to a possible COVID-19 infection before the first dose of the vaccine and within 14–21 days after the second dose of the vaccine. Blood samples were collected at the pre-defined time points. Sera were retrieved *via* centrifugation and stored at  $-80^{\circ}\text{C}$ .

Sera were tested for anti-SARS-CoV-2 receptor binding domain (RBD) spike protein IgG (anti-RBD), using the Abbott SARS-CoV-2 IgG II Quant assay (Abbott Laboratories, Abbott Park, IL, USA), which is a two-step chemiluminescent microparticle immunoassay intended for the qualitative and quantitative detection of IgG antibodies against the RBD of the S1 subunit of the spike protein in human serum and plasma on the Architect i system. The amount of IgG antibodies to SARS-CoV-2 in each sample is determined by comparing its chemiluminescent relative light unit (RLU) with the calibrator RLU (index S/C). The linear range is between 21 and 40,000 AU/ml. The method has a high clinical sensitivity [98.81% (95% confidence interval (CI), 93.56%–99.94%)] and specificity [99.55% (95% CI, 99.15%–99.76%)] in samples collected after 15 days after the positive polymerase chain reaction, at a cutoff value of 50 AU/ml.<sup>3</sup>

The correlation coefficient in weighted linear regression of World Health Organization (WHO) standard with the Abbott anti-RBD is 0.999, and transformation of Abbott anti-RBD AU/ml to WHO BAU/ml is feasible using the equation  $\text{BAU/ml} = 0.142 \times \text{AU/ml}$  [25]. The assay threshold of 50 AU/ml was defined as the seroconversion cutoff in this study.

### Safety follow-up

Local or systemic adverse events (AE) along with antipyretic or analgesic medication use within 7 days after each dose of the vaccine were recorded. Moreover, the patients were followed during the following 2 months for late AEs. The AEs were captured during the postvaccination sample collection visit, as well as during a phone call or visit, 2 months postvaccination. The patients were specifically inquired about local (pain, edema) or systematic (fever, malaise, headache) AEs, as well as about the use of antipyretic or analgesic medication during the first week after each dose.

The study was approved by the Institutional Review Boards of all three participating centers (Laikon General Hospital, Athens, Greece, 01/22/2021; Attikon Hospital, Athens, Greece, 02/24/2021; and G. Gennimatas General Hospital, Athens, Greece, 02/18/2021). The reporting of this study conforms to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.<sup>19</sup>

### Statistical analysis

All statistical analyses were conducted using the IBM SPSS statistics, version 26 (IBM Corporation, North Castle, NY, USA). The Pearson chi-square test was used to test for associations between categorical variables (Fisher's exact test being used when less than five patients in each category), the independent-samples Mann–Whitney *U* test for testing between a categorical variable with two levels and not normally distributed continuous variables, and the Kruskal–Wallis *H* test for categorical variables with more than two levels. Logistic regression analysis was performed in order to assess the association between categorical variables and the seroconversion rate, including variables that proved to be statistically significant in the univariate analysis. The level of significance for all statistical tests was set at a probability value lower than 5% (two-sided,  $p < 0.05$ ).

### Results

Sixty-one patients with CLL were included in the study. The main epidemiologic and clinical characteristics of the patients are shown in Table 1. All patients were vaccinated with the BNT162b2 mRNA Covid-19 vaccine and had two measurements of antibodies as per protocol, one before the

**Table 1.** Epidemiologic and hematologic characteristics of the patients..

Characteristic	Result
Number of patients, <i>N</i> (%)	61 (100)
Sex (male/female), <i>N</i> (%)	28/33 (45.9/54.1)
Age (years), median (range)	61 (41–88)
Disease duration (months), median (range)	83.0 (0.3–285)
Stage (RAI), <i>N</i> (%)	
0	16 (26.2)
1	28 (45.9)
2	6 (9.8)
3	5 (8.2)
4	6 (9.8)
Hemoglobin level (g/dl), median (range)	12.5 (9.0–16.7)
Lymphocyte count ( $\times 10^9/L$ ), median (range)	10.30 (0.25–209.00)
Neutrophil count ( $\times 10^9/L$ ), median (range)	4.00 (0.34–26.91)
Monocyte count ( $\times 10^9/L$ ), median (range)	0.66 (0.03–9.39)
Platelet count ( $\times 10^9/L$ ), median (range)	167 (52–345)
Gamma-globulin level (g/dl), median (range)	7.5 (1.2–27.5)
CRP level (mg/L), median (range)	1.7 (0.1–16.0)
CRP, C-reactive protein.	

first and one after the second dose of the vaccine. Prevacination samples were obtained within a median time of 2.5 (range, 0–5) days before the first dose of the vaccine, while postvaccination samples were obtained within a median time of 14 (range, 13–21) days after the second dose of the vaccine.

#### *Immunogenicity/seroconversion results*

The median postvaccination antibody titer in the whole cohort was 28.0 AU/ml (range, 0.0–40.000). The postvaccination antibody titers of 27 (45.0%) patients were above the threshold of 50 AU/ml, while the remaining 33 (55.0%) patients had titers lower than 50 AU/ml and were considered as not achieving seroconversion. Table 2 presents the results on the immunogenicity of the vaccine.

In one patient, the prevaccination antibody titer was above 50 AU/ml (557.6 AU/ml), a sign of previous infection, although he had not reported symptoms attributable to COVID-19 during the previous 12 months or a positive test for SARS-CoV-2 before the first dose of the vaccine. This patient was excluded from the immunogenicity analysis. It should be noted though that the patient was a 78-year-old female with a long history of CLL, previously treated with anti-CD20 antibody-based regimens and ibrutinib, and actively treated with venetoclax. The patient achieved an antibody titer of 40,000 AU/ml after the vaccination.

Among the studied variables shown in Table 2, the postvaccination immunization status was correlated with RAI stage (0–2 *versus* 3–4; 51.0% *versus* 18.3%,  $p=0.047$ ), the treatment status (treatment naïve, 63.0%; previously treated, 40.0%; actively treated patients, 26.1%,  $p=0.031$ ), the number of previous treatment lines (0–2 *versus* >2; 55.3% *versus* 8.3%,  $p=0.004$ ), and the platelet count ( $<100 \times 10^9/L$  or  $\geq 100 \times 10^9/L$ ; 52.9% *versus* 10.0%,  $p=0.015$ ) of the patients. Nevertheless, in a multivariate analysis model comprising the above four variables, none retained its statistical significance as a predictor of immunogenicity. There was also a non-statistically significant trend for lower gamma-globulin levels in patients not achieving seroconversion but, at the same time, a statistically significant linear positive relationship between the gamma-globulin level and the antibody titer ( $r=0.182$ ,  $p=0.046$ ). It should be noted that none of the patients had been treated with intravenous immune globulin during the study period or within 90 days before the collection of the first sample. In treatment naïve patients, there was no correlation of gamma-globulin levels with seroconversion, but seroconversion was correlated with higher gamma-globulin levels in previously/actively treated patients (6.2 g/dl in patients not achieving seroconversion *versus* 9.3 g/dl in patients achieving seroconversion,  $p=0.034$ ). Finally, a statistically significant linear positive relationship between the platelet count and the antibody titer was found ( $r=0.277$ ,  $p=0.002$ , Figure 1).

Treatment naïve patients had a significantly higher antibody titer than previously or actively treated patients (median, 950.7 AU/ml *versus*

**Table 2.** Immunogenicity of the BNT162b2 mRNA Covid-19 vaccine in patients with CLL.

Parameter	Seroconversion (50 IU/L)		<i>p</i>
	Yes	No	
Number of patients, <i>N</i> (%)	27 (45.0)	33 (55.0)	NA
Sex, <i>N</i> (%)			0.176
Male	10 (16.7)	18 (30.0)	
Female	17 (28.3)	15 (25.0)	
Age (years), median (range)	75 (41–88)	72 (60–86)	0.789
Stage (RAI), <i>N</i> (%)			0.130
0	11 (18.3)	5 (8.3)	
1	11 (18.3)	16 (26.7)	
2	3 (5.0)	3 (5.0)	
3	1 (1.7)	4 (6.7)	
4	1 (1.7)	5 (8.3)	
RAI stage high versus low, <i>N</i> (%)			0.047
High (3, 4)	2 (3.3)	9 (15.0)	
Low (0, 1, 2)	25 (41.7)	24 (40.0)	
Disease duration (months), median (range)	74.0 (4–123)	99.0 (3–153)	0.318
Treatment status, <i>N</i> (%)			0.009
Treatment naïve	17 (28.3)	10 (16.7)	
Previously treated	4 (6.7)	6 (10.0)	
Actively treated	6 (10.0)	17 (28.3)	
Previous treatment, <i>N</i> (%)			0.011
Yes	10 (16.7)	23 (38.3)	
No	17 (28.3)	10 (16.7)	
Previous treatment lines, <i>N</i> (%)			0.004
0	16 (26.7)	9 (15.0)	
1	2 (3.3)	9 (15.0)	
2	7 (11.7)	3 (5.0)	
3	0 (0.0)	6 (10.0)	
4	2 (3.3)	6 (10.0)	

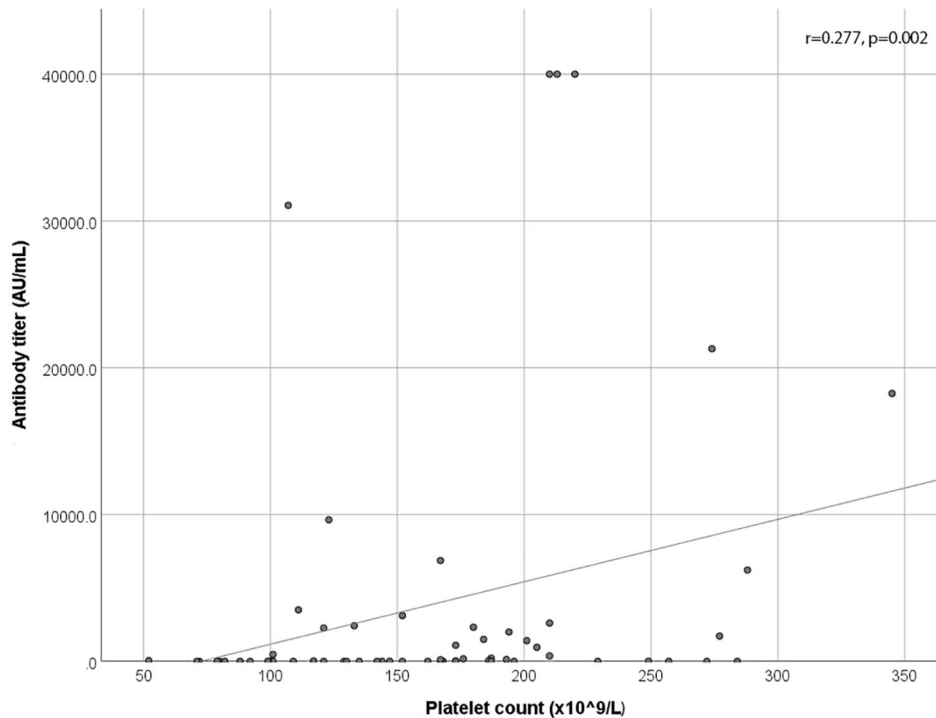
*(continued)*

**Table 2.** (continued)

Parameter	Seroconversion (50 IU/L)		<i>p</i>
	Yes	No	
Previous treatment lines, <i>N</i> (%)			0.004
>2	1 (1.7)	11 (18.3)	
≤2	26 (43.3)	21 (35.0)	
Previous anti-CD20 treatment, <i>N</i> (%)			0.234
Yes	6 (10.0)	12 (20.0)	
No	21 (35.0)	21 (35.0)	
Previous immunochemotherapy, <i>N</i> (%)			0.367
Yes	1 (1.7)	4 (6.7)	
No	26 (43.3)	29 (48.3)	
Previous ibrutinib, <i>N</i> (%)			1.000
Yes	1 (1.7)	1 (1.7)	
No	26 (43.3)	32 (53.3)	
Actively treated, <i>N</i> (%)			0.020
Yes	6 (10.0)	17 (28.3)	
No	21 (35.0)	16 (26.7)	
Hb (g/dl), median (range)	13.1 (10.4–16.0)	12.6 (9.0–16.7)	0.127
Lymphocyte count (×10 <sup>9</sup> /L), median (range)	10.3 (0.7–117.0)	5.2 (0.3–209.0)	0.602
Neutrophil count (×10 <sup>9</sup> /L), median (range)	3.6 (0.3–6.3)	4.1 (1.7–26.9)	0.839
Monocyte count (×10 <sup>9</sup> /L), median (range)	0.64 (0.03–5.66)	0.91 (0.22–9.39)	0.460
Platelet count (×10 <sup>9</sup> /L), median (range)	184 (52–288)	130 (71–284)	0.010
Gamma globulin (g/dl), median (range)	8.9 (4.6–20.2)	6.2 (1.4–20.8)	0.066
Active treatment with ibrutinib, <i>N</i> (%) <sup>a</sup>			0.621
Yes	1 (4.3)	6 (26.1)	
No	5 (21.7)	11 (47.8)	
Active treatment with venetoclax, <i>N</i> (%) <sup>a</sup>			0.283
Yes	4 (17.4)	7 (30.4)	
No	2 (8.7)	10 (43.5)	

CLL, chronic lymphocytic leukemia.  
<sup>a</sup>Among 23 actively treated patients during vaccination.





**Figure 1.** Linear correlation of platelet count and anti-SARS-CoV-2 antibody titer.

14.3 AU/ml *versus* 1.5 AU/ml,  $p=0.003$ , Figure 2), and the immunization/seroconversion rate was higher in treatment naïve *versus* previously or actively treated patients (63.0% *versus* 40.0% *versus* 26.1%, respectively,  $p=0.009$ ).

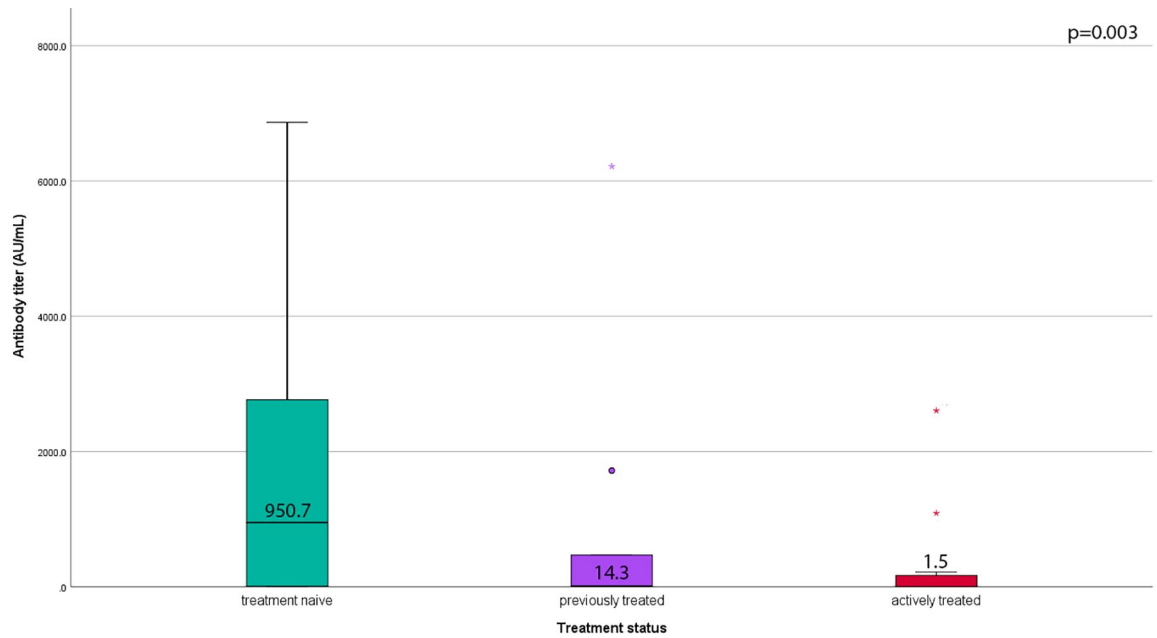
Finally, among actively treated patients ( $N=23$ , ibrutinib, 7; venetoclax, 11), although there was only a non-statistically significant trend for higher seroconversion rates in patients treated with venetoclax *versus* those treated with ibrutinib (36.4% *versus* 14.3%,  $p=0.308$ ), patients under treatment with venetoclax had higher antibody titers than those under treatment with ibrutinib (15.8 AU/ml *versus* 0.0 AU/ml,  $p=0.047$ , Figure 3).

### Safety results

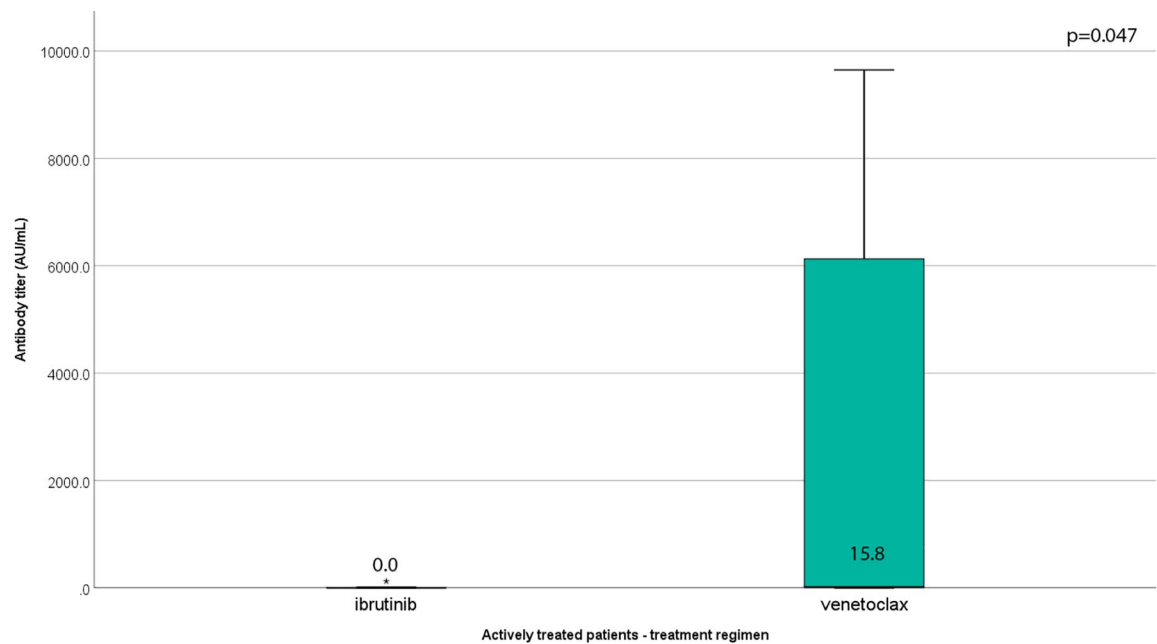
After the first dose of the vaccine, 53 (86.9%) patients reported no AEs. Among the remaining eight (13.1%) patients, five had only local reactions while three had low-grade fever lasting less than 48 h. After the second dose of the vaccine, 43 (70.5%) patients reported no AEs. Among the remaining 18 (29.5%) patients, 11 had only local reactions while 7 had systematic AEs (4, low-grade fever; 3, headache). Grade  $>1$  AEs were

not reported. All AEs emerged within 0–7 days from the administration of the vaccine. No late AEs were reported.

Furthermore, there was no correlation between the emergence of AEs after the first or second dose and the immunogenicity of the vaccine ( $p=0.803$  and  $p=0.123$ , respectively), although there was a marginally statistically significant correlation of the emergence of systematic AEs (i.e. fever) after the second dose of the vaccine with the antibody titer ( $p=0.051$ ). Regarding the correlation of AEs with the age or the sex of the patients, women tended to report more AEs than men after the second dose ( $p=0.066$ ), and younger patients tended to report more AEs than older ones after the first ( $p=0.066$ ). This difference was more pronounced after the second dose (median age of patients reporting an AE is 67.5 years *versus* 76.0 years for patients not reporting an AE,  $p=0.016$ ). Moreover, there was no correlation of AEs with RAI stage; previous treatment; number of previous treatment lines; previous treatment with anti-CD20 antibodies, ibrutinib, or fludarabine; or the hematologic parameters of the patients. Finally, regarding the emergence of AEs, there was no difference



**Figure 2.** Anti-SARS-CoV-2 antibody titers in patients with CLL per treatment status (treatment naïve, previously treated, and actively treated patients). Median values for each group are shown in the graph.



**Figure 3.** Anti-SARS-CoV-2 antibody titers in patients with CLL actively treated with ibrutinib or venetoclax. Median values are shown for both treatment groups in the graph.

between actively treated and untreated patients at the time of vaccination or the treatment regimen. Reporting an AE after the first dose was correlated with disease duration (long-standing disease was correlated with less AEs: 97.0 months

for patients reporting no AEs *versus* 51.0 months for patients reporting AEs,  $p = 0.043$ ). There was no correlation of the emergence of systematic AEs (i.e. fever) with any of the above studied factors.



## Discussion

Antibody-mediated responses in patients with CLL are usually modest because the disorder is characterized by inherent humoral and cell immunity defects while CLL-directed treatment may further diminish response to vaccinations. Recent studies have shown lower anti-SARS-CoV-2 antibody response rates in CLL patients vaccinated with anti-SARS-CoV-2 vaccines, particularly among those actively treated.<sup>20–22</sup>

In this study, low seroconversion rates were noted in patients with CLL vaccinated with two doses of the BNT162b2 mRNA Covid-19 vaccine. Only 45% of the patients achieved seroconversion, and this rate was comparable with that of previous studies.<sup>20,21</sup>

A higher RAI stage, active treatment, more than two previous treatment lines, and low platelet counts were all associated with poorer seroconversion rates. All these parameters are markers of advanced disease and have been found to be consistently correlated with profound immunosuppression.<sup>17,23,24</sup>

According to our results, in comparison to previously treated patients or actively treated patients, treatment-naïve patients were better responders to vaccination. The humoral immunity of this group may not be compromised to the same extent because no immunosuppressive treatment drugs are administered. However, even treatment-naïve patients are poorer responders to the vaccine compared with healthy adults because only 63.0% of the patients achieved seroconversion, a result that has also been demonstrated in other studies.<sup>20,22</sup> Moreover, previously treated patients showed a better response to the vaccine than actively treated patients. Consistently, previous reports documented a profound impact of active treatment on immunogenicity.<sup>20,22</sup>

Hypogammaglobulinemia is a cardinal feature of CLL. Emerging from early stages, despite initial low tumor load, with its severity increasing with disease progression, it has been correlated with higher infection risk and inadequate response to vaccination.<sup>7,24</sup> In addition, traditional chemotherapy and chemoimmunotherapy regimens tend to rather decrease serum immunoglobulin levels, thus exacerbating preexisting immunosuppression. Furthermore, a negative association of hypogammaglobulinemia with anti-SARS-CoV2

IgG development in CLL patients after COVID-19 disease was recently demonstrated.<sup>20</sup> In our study, there was a linear correlation of gamma-globulin levels with the antibody titers, as well as a lower gamma-globulin level in patients not achieving seroconversion. These results are in accordance with the results of previous reports.<sup>21,22</sup>

Regarding nontreatment naïve patients, we observed that patients previously or actively treated with ibrutinib, a BTKi used widely in CLL, achieved very low seroconversion rates. It is well known that BTKis inhibit the B-cell receptor signaling in all B-cells, both malignant and normal, thus provoking serious deregulation of the humoral immunity. In agreement with our results, recent studies reported reduced seroconversion, following SARS-CoV-2 vaccination, among patients under treatment with ibrutinib.<sup>21,25,26</sup> Moreover, earlier studies further support these findings documenting higher rates of seroconversion to recombinant hepatitis B and influenza vaccines in treatment-naïve patients as compared with patients treated with BTKis.<sup>13,25</sup>

Although in several studies it has been demonstrated that treatment with anti-CD20 antibodies has a profound impact on the immunogenicity after vaccination against SARS-CoV-2,<sup>20,21</sup> in our study, such results were not confirmed possibly because of the fact that none of the actively treated patients was under treatment with an anti-CD20 antibody. Anti-CD20 monoclonal antibodies lead to sustained B-cell depletion and hypogammaglobulinemia lasting for up to 6 months, with complete recovery of B-cells achieved, in the vast majority, after 1 year.<sup>27</sup>

Monotherapy with venetoclax, as demonstrated by our findings, seems to be less implicated in impaired immunization. Currently, the impact of venetoclax on humoral immunity remains unclear although studies of patients receiving regimens that contain the drug showed reduced numbers of nonmalignant B-lymphocytes.<sup>28</sup> Contrary to our results, Herishanu *et al.*<sup>21</sup> reported lower seroconversion rates among patients treated with venetoclax; however, a combination with an anti-CD20 agent was used in most of them while, in our study, all patients treated with venetoclax were receiving the drug as monotherapy. Interestingly, one patient who tested seropositive prior vaccination, implying previous natural infection, achieved exceedingly high antibody titers after the vaccination while

under treatment with venetoclax. Comparably, a superior response among previously infected CLL patients has been recently reported, implying a ‘vaccine-priming’ effect of previous natural infection while it is possible that immune memory prevails over the seroconversion failure.<sup>22</sup>

Concerning reactogenicity, no AEs were reported in the greatest proportion of patients following both vaccine doses. It should also be noted that no grade >1 AEs were recorded, implying that the vaccine is well tolerated by patients with CLL. Quite importantly, no correlation was observed between the emergence of an AE and optimal seroconversion, which is in accordance with the results stated by a previous study.<sup>20</sup> These findings indicate that the emergence of a side effect, systemic or local, is not a predictive factor of a positive response in patients with CLL. Younger and female patients inclined toward more frequent vaccination reactions, consonant with preceding findings, while disease stage or treatment status, seem not to correlate with the triggering of a reaction.<sup>20</sup> On the contrary, another study showed a correlation between the AE rate and the antibody titer in a cohort of hematological and oncological patients.<sup>29</sup> Nevertheless, it should be noted that patients with CLL comprised a small fraction of that cohort. Significantly, fewer reactions were observed among patients with long-standing disease, maybe explained by habituation of disease or treatment-related symptoms, thus failing, or neglecting, to recognize new ones.

The strengths of this study are the use of a homogeneous population of patients with CLL and only one type of anti-SARS-CoV-2 vaccine, as well as the analysis of prognostic and treatment-related factors correlating to vaccination efficacy. Considerably, in contrast to earlier reports, this study included several patients receiving monotherapy with venetoclax and demonstrated that this agent may be implicated at a lower degree in dampened immune responses to the vaccine. The main limitations of this study are the relatively small patient sample and the lack of measuring vaccine-induced T-cell immunity. Nevertheless, to our knowledge, no previous reports of SARS-CoV-2 vaccine impact on eliciting a T-cell response in CLL patients have been published, highlighting the need for further postvaccination T-cell immunity studies. It should be noted that it is possible that patients without seroconversion are to some extent protected against COVID-19

through T-cell immune responses. Moreover, we did not encompass a control group, because previous trials had already documented that CLL patients are less responsive to SARS-CoV-2 vaccination, in comparison to healthy individuals.<sup>20,22</sup> Finally, although the anti-RBD testing results are strongly correlated with the neutralizing antibody titers,<sup>30</sup> there is no formal correlation with protection because no threshold level has been established so far.

In conclusion, patients with CLL demonstrated suboptimal responses to SARS-CoV-2 vaccination with two doses of the BNT162b2 mRNA Covid-19 vaccine. Therefore, they should adhere to safety precautions and keep up social distancing while vaccination of family members is highly recommended. Vaccination protocols should also be adapted to the needs of CLL patients. Thus, patients should be vaccinated before treatment with highly immunosuppressive regimens, such as anti-CD20 antibodies and BTKi, a strategy that is feasible in many cases. Alternatively, vaccination of patients treated with anti-CD20 antibodies should be postponed for at least 6 months after the end of treatment. Nonetheless, proper vaccination strategies should be instituted, customized to each patient’s treatment plan. Because post-vaccination antibody durability is not known, an additional booster dose of the vaccine should be considered for all CLL patients, in order to maintain their serologic response. Whether a third booster dose could generate a late seroconversion in previously nonresponding CLL patients, as it was observed in solid organ transplant recipients, remains to be seen.<sup>31,32</sup> Alternating vaccine types could also contribute to seroconversion, although this strategy has not yet been supported by any trials. Finally, a temporary shift in our treatment strategies in patients with CLL could be beneficial, especially because many types of treatment regimens are now available. Hence, avoidance of anti-CD20 antibodies during the pandemic and replacing them with newer less immunosuppressive agents is an option in selected patients. Furthermore, according to our study, venetoclax could be a better candidate for targeted therapy compared with BTKi in patients with CLL although this finding has to be confirmed in larger, prospective studies. Henceforward, more prospective studies need to be conducted in an attempt to optimize SARS-CoV-2 vaccination efficacy in this vulnerable population until herd immunity is accomplished.

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## Conflict of interest statement


The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Data availability

Data on the experiments as well as patient data are available upon reasonable request.

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