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RESEARCH ARTICLE

# SARS-CoV-2 humoral responses following booster BNT162b2 vaccination in patients with B-cell malignancies

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## Abstract

Patients with B-cell malignancies have suboptimal immune responses to SARS-CoV-2 vaccination and are a high-risk population for severe COVID19 disease. We evaluated the effect of a third booster BNT162b2 vaccine on the kinetics of anti- SARS-CoV-2 neutralizing antibody (NAbs) titers in patients with B-cell malignancies. Patients with NHL ($n = 54$) Waldenström's macroglobulinemia ($n = 90$) and chronic lymphocytic leukemia ($n = 49$) enrolled in the ongoing NCT04743388 study and compared against matched healthy controls. All patient groups had significantly lower NAbs compared to controls at all time points. 1 month post the third dose (M1P3D) NAbs increased significantly compared to previous time points (median NAbs 77.9%, $p < .05$ for all comparisons) in all patients. NAbs ≥ 50% were seen in 59.1% of patients, 34.5% of patients with suboptimal responses post-second dose, elicited a protective NAb titer ≥50%. Active treatment, rituximab, and BTKi treatment were the most important prognostic factors for a poor NAb response at 1MP3D; only 25.8% of patients on active treatment had NAbs ≥ 50%. No significant between-group differences were observed. Patients with B-cell malignancies have inferior humoral responses against SARS-CoV-2 and booster dose enhances the NAb response in a proportion of these patients.

## 1 | INTRODUCTION

Effective and safe vaccine development against SARS-CoV-2 is imperative to the strategic management of the COVID-19 pandemic at a population and individual level.[1] Patients with hematological malignancies are not only at increased risk of severe COVID19 disease and worse outcomes[2,3] but also at increased risk of serological non-response to vaccination.[4] Recent data in patients with chronic lymphocytic leukemia (CLL), Non-Hodgkin's lymphoma (NHL), and Waldenström macroglobulinemia (WM) CLL, NHL, and WM patients report less effective humoral responses following vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as reflected by low titers of neutralizing antibodies (NAbs)[5,6] Being on active treatment, particularly with anti-CD20 monoclonal antibodies, Bruton's Tyrosine Kinase inhibitors and B-cell lymphoma 2 inhibitors, has emerged as the main negative prognostic factor for suboptimal antibody response in these.[5–7]

Vaccination has lowered the risk of severe COVID-19 disease significantly among immunocompetent, and immunocompromised individuals, despite suboptimal humoral responses among the latter.[4] The emergence of new SARS-CoV-2 variants and the declining humoral immunity over time[8] have necessitated the administration of booster vaccine doses.[9,10] Recent data have demonstrated increased antibody titers and no adverse toxicities following a third booster dose in immunocompetent and immunocompromised

  

patients.[11–13] Given the need to maximize the protection of hematological patients against SARS-CoV-2 and to enhance immune responses the Advisory Committee of Immunization Practices and the CDC were prompted to recommend a booster shot of COVID-19 vaccines, in immunocompromised patients.

Initial humoral response data following vaccination against SARS-CoV-2 in patients with hematological malignancies have therefore questioned the ability of these patients to elicit satisfactory humoral responses and establish adequate antibody titers.[14] In this context we evaluated prospectively, following up on previously reported data, the development of NAbs against SARS-CoV-2 in patients with CLL, NHL, and WM up to 30 days postvaccination with a third booster dose of the messenger RNA BNT162b2 vaccine (registered at www.clinicaltrials.gov as #NCT04743388).

## 2 | METHODS

### 2.1 | Clinical study

All participants have been enrolled in a large prospective study (NCT04743388) evaluating the kinetics of anti-SARS-CoV-2 antibodies after COVID-19 vaccination in healthy subjects and patients with hematological malignancies or solid tumors. According to the National Vaccination Program in Greece, the first two doses of BNT162b2 are administered within 3 weeks. Patients with hematological malignancies had a third booster dose at least 3 months after and up to 6 months following the second vaccine dose. Healthy subjects received the third booster dose 6 months after the second vaccine dose. The study was approved by the Institutional Ethics Committee of General Hospital Alexandra, Athens, Greece in accordance with the Declaration of Helsinki and the International Conference on Harmonization for Good Clinical Practice. All patients and controls provided written informed consent prior to enrollment in the study. In compliance with the General Data Protection Regulation, data confidentiality was strictly protected.

Major inclusion criteria for the study included: age above 18 years; diagnosis of NHL, CLL, and WM irrespective of the treatment phase; and eligibility for vaccination. Volunteer healthy individuals without malignant disease of similar age were also included in this analysis as controls. Major exclusion criteria for both patients and controls included the presence of autoimmune disorders or active malignant disease besides CLL, NHL, WM; HIV or active hepatitis B and C infection, and end-stage renal disease. These entities were excluded due to concerns of confounding effect on antibody response following vaccination.

Relevant data were extracted from the medical records and included: demographics, medical history, symptoms from the disease, medication, complete blood count, serum immunoglobulin (Ig) levels, disease status, and type of treatment. Body mass index (BMI) was computed using the individual's weight and height.

### 2.2 | Antibodies measurement and data collection

The blood collection schedule for this clinical investigation was as follows: on day 1 (D1) before the first vaccination, at 3 weeks (i.e., day 22 prior to the second dose), 1 month (D50), and 3 months (3 M) post second dose, and 1 month post the third vaccination (1MP3D). An additional sampling point was scheduled for patients; this was set for the day of the third dose, prior to the vaccination.

Blood was collected and serum was extracted within 4 h of collection. The serum was subsequently stored at −80°C until the day of the measurement. NAbs against SARS-CoV-2 were analyzed utilizing an FDA-approved method. The cPassTM SARS-CoV-2 NAbs Detection Kit from GenScript (GenScript, Inc.; Piscataway, NJ, USA) was used in this investigation to detect SARS-CoV-2 NAbs in blood in an indirect[15] manner as described previously.[16,17] Samples of the same individual were measured in the same ELISA plate. A NAb titer of at least 30% is considered as positive, whereas a NAb titer of at least 50% has been associated with clinically relevant viral inhibition.[18]

### 2.3 | Data analysis

Statistical analysis began with descriptive criteria such as mean, median, quartiles, and estimation of dispersion metrics. To determine the normality of the data distribution, the Shapiro–Wilk test was used. If the nominal normality hypothesis is rejected, it is assumed that the variable does not follow the normal distribution. The variables (i.e., the values of neutralizing antibodies in different time periods) were found to deviate from the normal distribution in all cases of this study. For this reason, nonparametric approaches were used in the analysis. The Mann–Whitney U test was used for comparisons between two independent groups, for example, for analysis of the age effect or the effect of BMI. The Wilcoxon signed-rank test was used for pairwise group comparisons, such as neutralizing antibody levels between two occasions. The Kruskal–Wallis test was used for simultaneous comparison of the three patient groups (NHL, CLL, WM). When this test showed statistical significance, the Van der Waerden post hoc test was used to identify the different groups. Finally, the Friedman nonparametric test was applied to detect differences between individuals (in their Nabs titers) across multiple time points, i.e., day 22, 50, etc. In all cases in this study, the significance level was set at 5% and a result was considered significant if the estimated $p$-value ($p$) was less than the significance level. The statistical analysis was implemented in IBM® SPSS® Statistics (version 26).

Apart from the classic statistics, Principal Component Analysis (PCA) was also applied to transform a high-dimensional set of features into a low-dimensional set of features and possibly unveil relationships among the characteristics. PCA converts the original space generated by the original dataset into a new space that is a linear combination of the dataset's dimensions. Each new dimension created is referred to as a principal component (PC). The new locations of the data are referred to as "scores." Each PC accounts for a part of the variation in the original data set. The first principal component's direction is the direction in which the data varies the most. The

contribution of each original dimension to the new dimension is defined using a set of parameters known as "loadings." Each principal component is a normalized linear combination of the original features, where normalized means that the squared sum of each principal component's loadings equals one. The closer the loading value is to +1 (or −1), the greater (or lesser) the contribution of that feature to principal component. PCA analysis was performed in Python v.3.9.2.

## 3 | RESULTS

### 3.1 | Demographics and clinical characteristics of the patients

A total of 193 patients who received three doses of BNT162b2 mRNA vaccine were included in the study; patients with NHL ($N = 54$), CLL ($N = 49$), and WM ($N = 90$). Each matching control group had the same sample size as the corresponding patient category. In these patients, NAbs were measured on day 1, 22, one, and three months after the second vaccination, on the day of the third dose (which took place 3–6 months following the second vaccine dose) and 1 month post the third dose. The demographic data of the subjects who participated in this study are shown in Table 1 (panel A). The median age of the entire patient cohort was 73 years, and similar proportions of men (47.2%) and women (52.8%) participated in the study. The corresponding age values for the control group were 72 years, and the proportions of men/women were 46.1% and 53.9%, respectively. With respect to each hematological malignancy group (NHL, CLL, WM) (Table 1 panel A) and the clinical characteristics of the participants (Table 1 panel B), similar numbers and proportions exist between the patients and the healthy control groups, so that the following analysis could be reliably performed.

**A. Characteristics of the study participants**

| | Patients | | | |
|---|---|---|---|---|
| **Characteristic** | **Entire patient group** | **NHL** | **CLL** | **WM** |
| Sample size | 193 | 54 | 49 | 90 |
| Men (n, %)) | 91 (47.2%) | 29 (53.7%) | 24 (49.0%) | 38 (42.2%) |
| Women (n, %) | 102 (52.8%) | 25 (46.3%) | 25 (51.0%) | 52 (57.8%) |
| Age (median, IQR) | 73 (15) | 71 (23) | 71 (9) | 76 (18) |
| BMI (median, IQR) | 26.2 (5.3) | 25.9 (6.7) | 26.6 (6.3) | 26.1 (5.1) |
| | **Healthy controls** | | | |
| **Characteristic** | **Entire control group** | **Control-1** | **Control-2** | **Control-3** |
| Sample size | 193 | 54 | 49 | 90 |
| Men (n, %)) | 89 | 28 (51.9%) | 23 (46.9%) | 38 (42.2) |
| Women (n, %) | 104 | 26 (48.1%) | 26 (53.1%) | 52 (57.8%) |
| Age (median, IQR) | 72 (20) | 71 (22) | 70 (8) | 75 (16.1) |
| BMI (median, IQR) | 26.1 (6.1) | 26.5 (7.2) | 26.9 (6.8) | 26.3 (5.3) |

**B. Clinical and treatment characteristics of patients**

| **Characteristic** | **Value (percentage)** | **Value (percentage)** |
|---|---|---|
| Symptomatic | No | 51 (26.6%) |
| | Yes | 142 (73.4%) |
| Active treatment | No | 127 (65.8%) |
| | Yes | 66 (34.2%) |
| Type of treatment (at vaccine timepoint) | BTKi-based | 27 (40.9%) |
| | Rituximab-based | 23 (34.9%) |
| | BTK-Ritux combination | 6 (9.1%) |
| | Chemotherapy only | 7 (10.6%) |
| | Venetoclax | 3 (4.5)% |

*Notes*: n, number of subjects; IQR, Inter-Quartile Range. Among patients with NHL: 53% were male, median age was 70 years, 80% had symptomatic disease, 37% were on active treatment and 37% had received Rituximab during the last 12 months. Among patients with CLL, 49% were male, median age was 73 years, 60% had symptomatic disease, 41% were on active treatment and 4% had received Rituximab during the last 12 months. Among WM patients, 43% were male, median age was 73 years, 78% had symptomatic disease, 33% were on active treatment and 20% had received Rituximab during the last 12 months.

**TABLE 1** Demographics, clinical and treatment characteristics of the study participants. Three groups of patients with hematologic malignancies were included in the study: patients with Non-Hodgkin Lymphoma, Chronic Lymphocytic Leukemia (CLL), and Waldenström macroglobulinemia (WM). Three relevant groups of healthy individuals were also included as matching controls (panel A). The study population was further analyzed in terms of exhibiting malignancy symptoms, receiving or not treatment during the study period, and the type of treatment

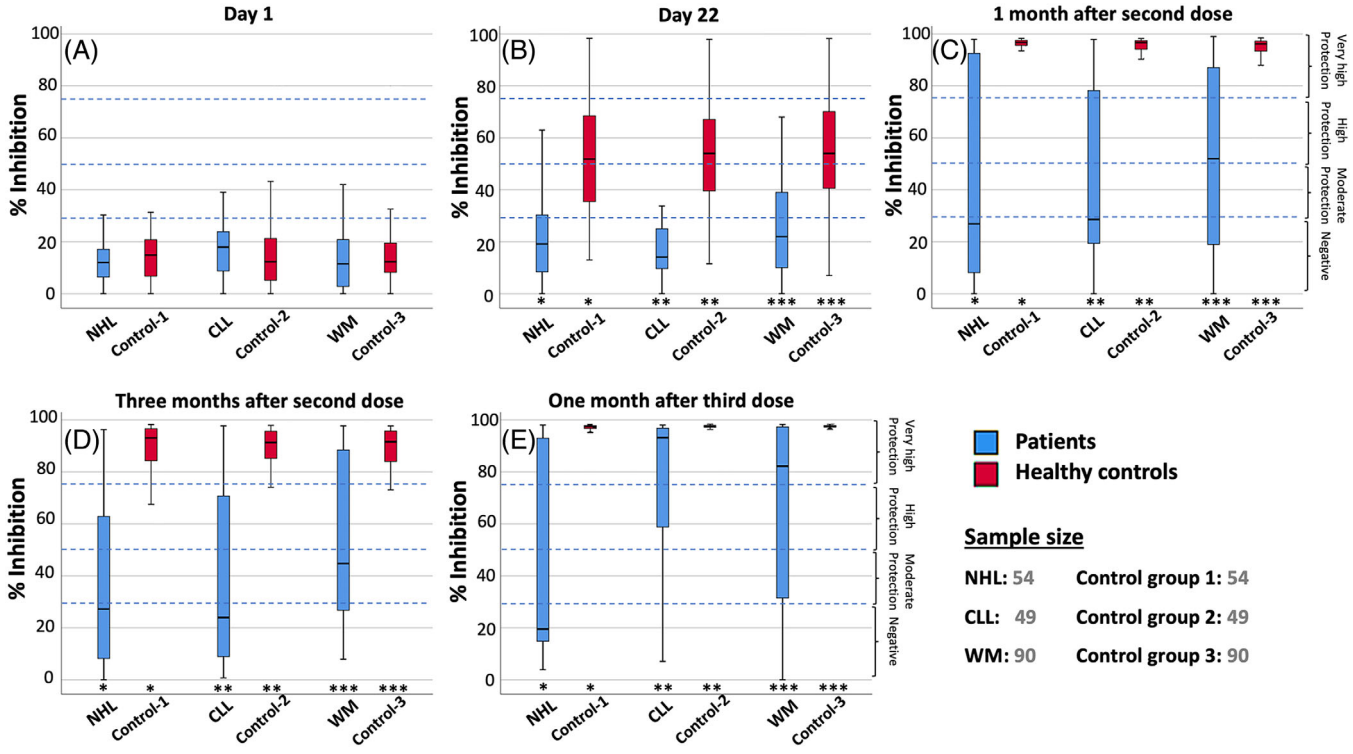


**FIGURE 1** Inhibition (%) of SARS-CoV-2 binding to the human host receptor angiotensin-converting enzyme-2 after vaccination with the BNT162b2 mRNA vaccine. Antibodies were measured on day 1 (A), day 22 (B), 1 month after the second dose (C), 3 months after the second dose (D), and 1 month after the third dose (E). Two groups of subjects participated in the study: patients with hematologic malignancies (NHL, CLL, WM) and the corresponding healthy controls (groups: 1, 2, 3). Asterisks (*, **, ***) indicate statistically significant differences (Mann–Whitney $p < 0.05$) between the compared groups. The same asterisk symbol is used for the groups being compared. The boundaries of the boxplot refer to the quartiles of the distribution, while the dashed lines of the graph indicate the limits of inhibition, i.e., 30%, 50%, and 75%

## 3.2 | Neutralizing antibodies

### 3.2.1 | Overall time profile

Figure 1 shows the percent inhibition of Nabs on days 1, 22, one and three months after the second dose and 1 month after the third booster dose in the three patient groups (NHL, CLL, WM) and the three corresponding control groups (1, 2, 3). On day 1, the median NAb titer was comparable across all groups ($p > 0.05$) (Figure 1A). On day 22, the NAb levels of all patients remained low and significantly lower ($p < .001$) compared to healthy controls (Figure 1B). Similar performance in inhibiting NAbs was observed across the three patient groups (overall median 18%) and in the three control groups (median 53.7%). 1 month after the second vaccination (Figure 1C), inhibition levels increased in all participants, but remained significantly lower ($p < .001$) in patients (median 35.9%) compared to healthy controls (median 96.4%). Of note is the wide dispersion of inhibitory values in each patient group (NHL, CLL, WM) compared with the very compressed values in the three healthy groups; indicatively, the calculated interquartile ranges of NAbs were approximately 75% for patients and only 3% for the healthy groups. The same motif was also observed 3 months after the second vaccination (Figure 1D), where a slight decrease in NAbs values was observed in all cases (both healthy and patients). Again, NAbs levels in patients were significantly ($p < .001$) lower than those of the healthy subjects.

Finally, 1 month post the third dose (Figure 1E), NAbs levels were very high in all healthy participants (median 97.5%), whereas in patients, NAbs levels increased significantly compared with levels at previous time points. The exception was the NAbs levels of the NHL group; even though they increased after the third booster dose, as depicted by the dispersion towards higher values, for half of the patients the NAbs levels remained less than 20% inhibition. Indeed, for the NHL group, there were 32 patients, namely 59.3% of NHL patients with NAbs levels less than 30% after the third dose.

Details of NAbs kinetics in all patients and each patient group can be seen in Figure 2. The median NAb B3D (before 3rd dose) was 23.5% for all patients and increased to 77.9%. The increase in median NAb titer B3D to M1P3D was significant in the CLL (34.1% vs 76.2% $p = .001$) and WM (25.3% vs 82.2%, $p < .001$) subgroups, but not the NHL subgroup (18.5% vs 31.6%, $p = .062$).

Among all patients, 23.5% had NAb≥50% B3D versus 77.9% at 1MP3D. The respective subgroup numbers for NAb ≥50% at 1MP3D were 81.3% for CLL, 60.6% for WM, and only 35.3% for NHL.

Thirty-four percent of patients who had NAbs <50% before the third dose (B3D), increased their NAb titer to ≥50% at 1MP3D. Approximately 34.5% of patients with a suboptimal response after the

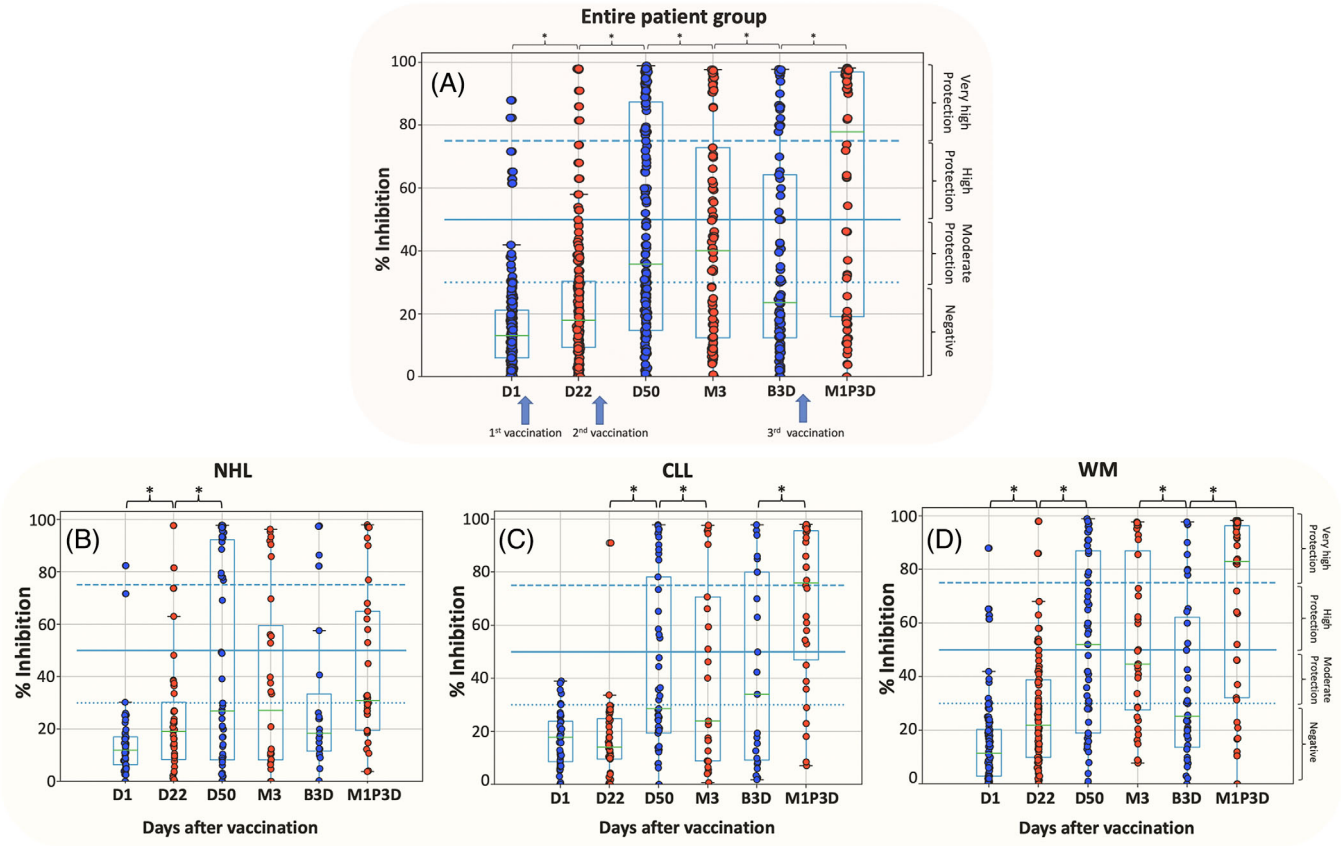


**FIGURE 2** Inhibition (%) of SARS-CoV-2 binding in all patients with hematologic malignancies (A) and each patient group (B: NHL, C: CLL, D: WM), after vaccination with the BNT162b2 mRNA vaccine. Antibodies were measured on day 1 (D1), day 22 (D22), 1 month after the second dose (D50), 3 months after the second dose (M3), immediately before the third dose (B3D), and 1 month after the third dose (M1P3D). The asterisk (*) indicates statistically significant differences (Wilcoxon $p < 0.05$) between the compared groups. The boundaries of the boxplot refer to the quartiles of the distribution, while the dashed lines of the graph indicate the limits of inhibition, i.e., 30%, 50%, and 75%. *Key*: NHL, non-Hodgkin lymphoma; CLL, Chronic lymphocytic leukemia; WM, Waldenström Macroglobulinemia

2nd dose, elicited an antibody titer ≥50% following the third booster dose. Finally, a comparison of neutralizing antibody levels between the three patient groups (NHL, CLL, WM) at all these time points revealed no statistically significant differences ($p > .05$).

### 3.2.2 | Therapy and disease effect

To investigate the effect of treatment on inhibition levels in patients, further analysis was performed (Figure 3). Figure 3A,B show NAbs levels prior to and 1 month post the third dose in patients were on active treatment compared to those not on active therapy. Before the third dose, inhibitory concentrations of NAbs were lower in all patient subgroups on active treatment ($p > .05$). Prior to the 3rd dose, across all patient groups on treatment, inhibitory levels were below the critical value of 30% in at least 75% of individuals. 1 month post the third dose (Figure 3B), inhibitory levels increased significantly in all patients not on active treatment, and in all these cases, median inhibitory levels were above 95%. In contrast, NAbs kinetics were different in patients under active treatment. Only 28% of patients on active treatment had NAbs ≥50% prior to the 3rd dose, which remained very low at 25% at 1MP3D. More specifically, no increase was observed in the patients with NHL and CLL. In the WM group, the increase in NAbs was almost 100%, i.e., an increase from median inhibition of 19% B3D to 41.2% at 1MP3D was observed (Figure 3B vs Figure 3A). At 1MP3D only 11% of patients with NHL, 25% with CLL and 41.2% with WM on active treatment had NAbs ≥50%. The differences between treated and non-treated patients were statistically significant among all patient subgroups ($p < .05$).

The role of rituximab treatment in the NHL and WM groups was explored further (Figure 3C,D). Immediately prior to the third dose, both NHL and WM patients who had received rituximab treatment within the last 12 months, had lower NAbs compared to patients who had not but the differences were not significant (Figure 3C). At 1MP3D, the effect of rituximab on the development of neutralizing antibodies, became quite evident (Figure 3D). Rituximab-treated NHL patients did not increase their NAbs levels (median NAbs 16% B3D vs 19% M1P3D) compared to rituximab-untreated NHL patients who showed a steep increase in NAbs, which reached a median value of 71.4% (from 44% B3D) ($p = .001$). Only 10% of NHL rituximab-

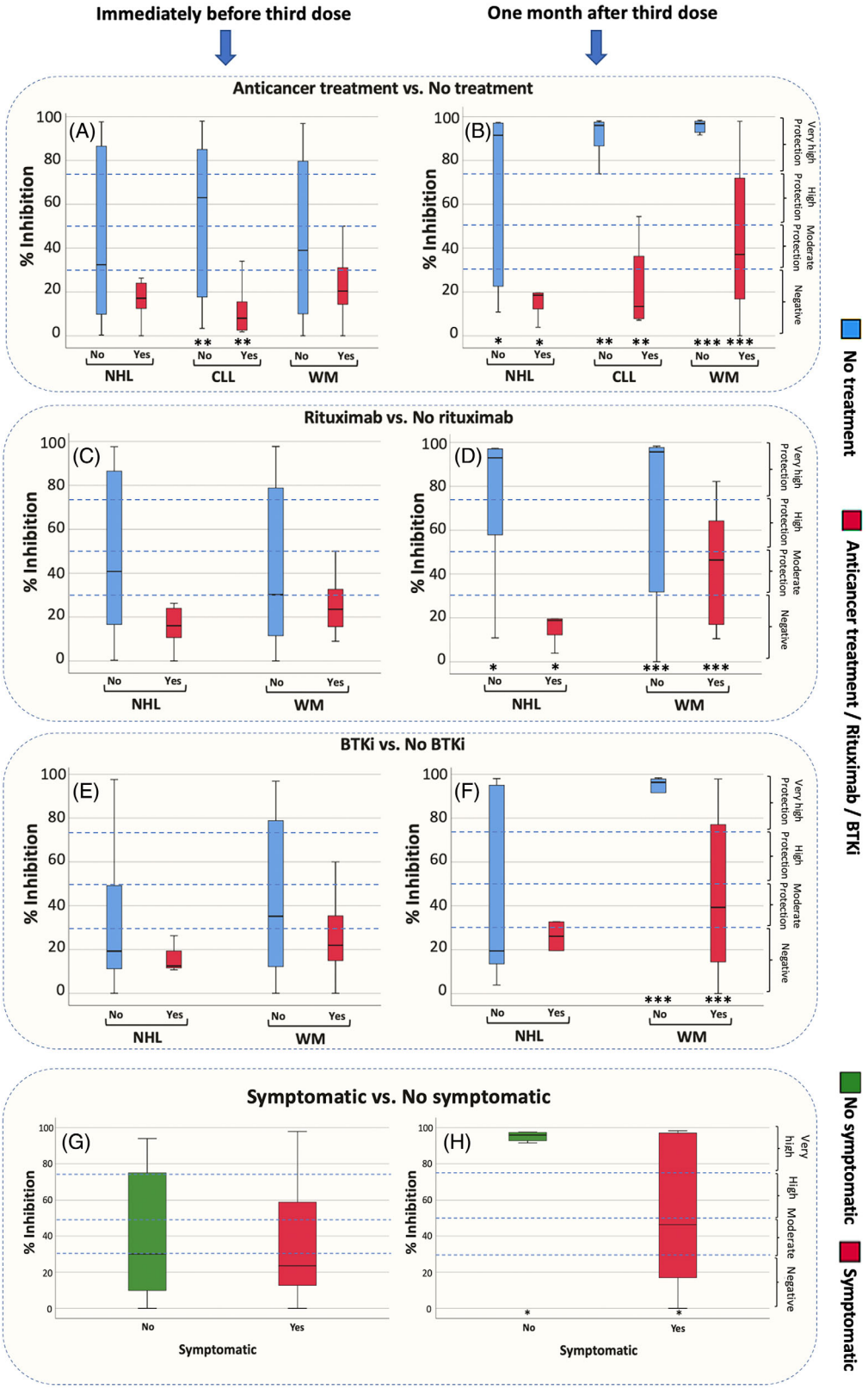


**FIGURE 3** Comparison of inhibition (%), of SARS-CoV-2 binding, immediately before the third booster dose and 1 month after vaccination with the BNT162b2 mRNA vaccine, in patients with Non-Hodgkin (NHL), chronic lymphocytic leukemia (CLL), and Waldenström Macroglobulinemia (WM). Four conditions were explored: Anticancer treatment (A) versus no treatment (B), Rituximab treatment (C) versus No rituximab treatment (D), BTKi treatment (E) versus no BTKi treatment (F), and symptomatic patients from the disease (G) versus no symptomatic (H). Asterisks (*, **, ***) indicate statistically significant differences between the compared groups. The same asterisk symbol is used for the groups being compared. The boundaries of the boxplot refer to the quartiles of the distribution, while the dashed lines of the graph indicate the limits of inhibition, i.e., 30%, 50%, and 75%

treatment patients had NAbs ≥50% following the third booster dose (vs 71.4% in rituximab untreated patients) WM patients not on rituximab also increased the NAb titer significantly (median 96% M1P3D) compared to rituximab-treated patients (46%) ($p = .040$).

We also assessed the effect of BTK inhibitors in the WM group; At M1P3D, the median NAb titer was 39% in BTKi-treated versus 96% in BTKi-untreated patients ($p = .003$) and the percentage of patients with NAbs ≥50% was 43.8% and 76.5% respectively (Figure 3E, F).

The levels of neutralizing inhibition, in response to vaccination, were also evaluated between symptomatic and asymptomatic patients due to disease status (Figure 3). Both groups had similar NAbs prior to the third vaccination dose (Figure 3G). However, the response was significantly different between the two groups 1 month post the third dose (Figure 3H). In asymptomatic patients, NAbs increased to very high levels (median 96%, 100% had NAb≥50%), while in symptomatic patients the median inhibition was 46.4%, NAbs ≥50% in 49.1% of patients ($p = .021$). For both groups of patients, the increase in NAbs following the third dose was statistically significant ($p < .001$).

### 3.2.3 | Relationship of neutralizing activity with age and BMI

To investigate the effect of age on antibody response, the patient cohort was split into two groups using the cut-off value of 71 years (i.e., the median age of the patients). Statistical comparison using the Mann–Whitney criterion revealed no significant differences in 1MP3D values between the two groups ($p = .781$). Similar analyzes were also performed for each patient group separately (i.e., NHL, CLL, WM), using the median age of each group as the cut-off value (see Table 1). Again, no statistically significant differences were found; $p$-values were 0.986, 0.845, and 0.798 for NHL, CLL, and WM, respectively.

The possible influence of BMI was also investigated in this way. The median BMI values of the whole patient population and of each group (NHL, CLL, WM) were used to divide the samples into two BMI groups. However, in all these cases, no statistically significant difference was found.

In addition, principal component analysis was used to extract information from the participants and analyze their relationship with NAbs scores. Figure S1A (in the Supplementary material) shows the results of PCA analysis applied to patients and Figure S1B shows the results applied to the corresponding healthy controls. The observations (patients or healthy controls) are represented as points in the plane generated by the two principal components, whereas the lines reflect the vectors of the variables, specifically the NAbs values on the measurement days, as well as age and BMI.

For patients (Figure S1A), the first two principal components explained 61.5% of the total variability (32.4% and 29.1% for the first and second components, respectively). Figure S1A shows that 1MP3D and D50 are quite close to each other on the right side of the graph near the first principal component, indicating a significant relationship. This suggests that someone with high NAbs on D50 is likely to have high NAbs on 1MP3D as well. With respect to the first principal component, the loading values of age and BMI are low, indicating that their influence on neutralizing antibody levels is rather small.

In healthy subjects (Figure S1B), the PCA plot is different. The two principal components explain 59.7% of the variability (38.5% and 21.2% for the 1st and 2nd principal components). With the exception of age and BMI, the neutralizing antibodies have a negative sign and are located on the left side of the graph. As for the first principal component, age has a positive charge value (0.42) and is located in the right part of the graph, meaning that it makes a negative contribution to NAbs values, i.e., as age increases, inhibitory activity decreases. However, the angle between 1MP3D and age is close to 90°, indicating a small effect of age on NAbs values 1 month after the third vaccination. The loading value of BMI with respect to the first principal component is almost zero (0.01), indicating that it makes a negligible contribution to the inhibition values. Moreover, there appears to be no relationship between 1MP3D and D22/D50 levels in healthy individuals.

## 4 | DISCUSSION

Our study demonstrates that a third BNT162b2 booster dose in patients with CLL, WM, and less so NHL, improves the humoral response against SARS-CoV-2, as reflected by an increase in NAbs 1 month following the booster dose (median NAb 77.9%1MP3D). Across all patient groups, approximately 34.5% of patients with a suboptimal response 1 month after the second dose, had a protective NAb titer of ≥50% 1 month following the booster dose. As expected, antibody titers were lower compared with controls of similar age and gender at all timepoints (NAbs ≥50% seen only in 59.1% at 1MP3D) as humoral immune responses are poorer in patients with underlying B-cell hematological malignancies.[5,6] At all timepoints there were no statistically significant differences in NAbs across patient groups.

Our results are in agreement with the improved humoral responses reported after a third BNT162b2 dose in solid-transplant patients[19] and patients with other hematological malignancies such as multiple myeloma, following suboptimal responses after the 2nd dose.[13] More specifically, all three patient groups showed a significant increase in the median NAb titer following the third dose. Some groups have reported meaningful increases in the antibody titer of previously seronegative patients with CLL and lymphoid malignancies following the third dose of the vaccine.[20–22] Other studies have however failed to demonstrate enhanced humoral immune responses in patients with B-cell malignancies who are seronegative after the second dose.[7,23,24]

Being on active treatment is the single most important adverse prognostic factor for a poor humoral response to the booster dose, which also explains the higher NAb titers among asymptomatic patients. Among patients on active treatment, only 8.7% of patients had NAbs ≥50% prior to the third dose, which remained very low at 25.7% at 1MP3D. In addition to active treatment, patients who had received Rituximab within the last 12 months and those who were on treatment with BTKi were unable to mount satisfactory immune responses. A recent report on 44 NHL patients also demonstrated that rituximab-based treatment within 6 months prevented effective immune responses to a third BNT162b2 vaccine dose.[25] Therefore, in agreement with previous reports, our data confirm that active treatment is the strongest adverse predictive factor with regards to low NAb titers at all timepoints, including following the third booster dose. B-cell depleting and/or immunomodulatory agents, such as anti-CD20 antibodies, BTK, and BCL2 inhibitors are in particular associated with suboptimal humoral responses.[26–30] There is multifactorial deregulation of the immune system associated with the underlying B-cell pathology and

the treatment effects.[31–34] A number of studies have demonstrated impaired immune responses to SARS-CoV-2 vaccination, even after a booster dose, in patients who receive anti-CD20 monoclonal antibodies and BTKi.[30] We do not report data for BTKi in CLL patients and BCL-2 inhibitor effects on NAb titers specifically, due to small patients' numbers within each group.

One of the main strengths of our study is the evaluation of neutralizing antibodies which are considered significant surrogates of vaccine efficacy. They have a high predictive value in terms of immune protection from COVID19 disease.[35,36] Our study is however limited by the lack of data regarding T-cell immune responses which reflect cellular immunity and could contribute partly to vaccine-effectiveness in this population. In a recent study, T-cell responses were assessed in CLL and NHL patients and dose 3 of the vaccine was found to restore cellular responses to comparable levels to healthy controls who had received 2 doses of the vaccine.[23] This finding needs to be investigated further as it could reflect a stimulating effect of the booster dose on the cellular immune response even in the absence of a humoral response.

Improved humoral responses have been linked to lower rates of infection and less severe disease presentation.[37] The clinical relevance and protective effects of the third booster dose on COVID19 hospitalization and death remain to be established with longer follow-up of the cohort. During the period of 1 month following the booster dose, no COVID19 cases were reported.

Declining humoral immunity over time and the emergence of new SARS-CoV-2 variants have necessitated the administration of booster vaccine doses. The running vaccination program in Greece recommends a third and a fourth booster vaccine against SARS-CoV-2 for immunocompromised patients and immunocompetent adults.[8] Testing NAbs titers following the third vaccine dose, could improve the selection and guide timing for high-risk hematological patients who are candidates for a 4th booster dose.

The optimal anti-COVID19 vaccination regimen in patients with B-cell hematological malignancies is yet to be determined. Booster doses seem to maintain declining NAb titers following the second dose. In a proportion of patients, particularly responders, it is possible to augment serologic responses with a booster dose. Future investigation and longer follow-up of ongoing trials will provide insight into the optimum utilization of vaccines against SARS-CoV-2.[38] Postvaccination COVID19 titer testing, remains an important part of managing these patients, as it can direct the timing and number of booster vaccinations.[39] Given the high-risk profile of hematological cancer patients and the overall suboptimal seroconversion rates, patients should be encouraged to use self-protective measures at all times and enroll in clinical trials.

## AUTHOR CONTRIBUTIONS

Conceptualization, **Evangelos Terpos**, **Ioannis P. Trougakos** and **Meletios A. Dimopoulos**: data curation, **Vangelis Karalis** and **Despina Fotiou**: formal analysis, **Vangelis Karalis**: funding acquisition, **Evangelos Terpos** and **Efstathios Kastritis**: investigation, **Despina Fotiou**, **Ioannis Ntanasis-Stathopoulos**, **Maria Gavriatopoulou**, **A.M.D.**, **Panagiotis Malandrakis**, **Vassiliki A. Iconomidou**, **Ioannis P. Trougakos** and **Efstathios Kastritis**: methodology, **Evangelos Terpos**, **Ioannis P. Trougakos**, **Maria Gavriatopoulou**, **I.P.**, **Despina Fotiou** and **Vangelis Karalis**: project administration, **A.M.D.**, **Vassiliki A. Iconomidou**, and **Panagiotis Malandrakis**: resources, **Maria Gavriatopoulou** and **Evangelos Terpos**: software, **Vangelis Karalis**: supervision, **Evangelos Terpos**, **Ioannis P. Trougakos**, **Efstathios Kastritis** and **Meletios A. Dimopoulos**: validation, Ioannis Ntanasis-Stathopoulos and Maria Gavriatopoulou; visualization, Vangelis Karalis; writing—original draft, **Evangelos Terpos**, **Vangelis Karalis** and **Despina Fotiou**: Writing—review and editing, **Maria Gavriatopoulou**, **Panagiotis Malandrakis** and **Meletios A. Dimopoulos**. All authors have read and agreed to the final version of the manuscript.

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## CONFLICTS OF INTEREST

The authors declare no relevant conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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