Serologic Response to mRNA COVID-19 Vaccination in Lymphoma Patients

Authors: Eric M. Jurgens[1], Thomas J. Ketas[2], Zhen Zhao[3], Michael J. Satlin[1, 3], Catherine B Small[1], Ashley Sukhu[3], Erik Francomano[2], PJ Klasse[2], Arcania Garcia[1], Emeline Nguyenduy[1], Erica Bhavsar[1], Silvia Formenti[4], Richard Furman[1], John P Moore[2], John P Leonard[1], Peter Martin[1].

Affiliations:

[1]Department of Medicine, Weill Cornell Medicine-New York Presbyterian Hospital-Weill Cornell Medical College, New York, NY

[2]Department of Microbiology and Immunology, Weill Cornell Medicine-New York Presbyterian Hospital-Weill Cornell Medical College, New York, NY

[3]Department of Pathology and Laboratory Medicine, Weill Cornell Medicine-New York Presbyterian Hospital-Weill Cornell Medical College, New York, NY

[4]Department of Radiation Oncology, Weill Cornell Medicine-New York Presbyterian Hospital-Weill Cornell Medical College, New York, NY

Corresponding author: Peter Martin, MD

Abstract word count: 173

Text word count: 3353

Tables: 1

Figures: 4

Short title: COVID-19 mRNA vaccine response in lymphoma

Keywords: COVID-19, lymphoma, BNT162b2, mRNA-1273

Data availability: All data requests should be sent to the corresponding author

Funding statement: This study was funded by the WCM Lymphoma Program, JPM, PJK, EF and TJK were supported by NIH grants R01 AI36082 and P01 AI110657

Ethics approval: This study was approved by the WCM IRB (IRB 21-02023288)

Patient consent: All patients provided written informed consent

Disclosures: PM has consulted for ADCT, AstraZeneca, Bayer, Beigene, BMS, Cellectar, Epizyme, Gilead, Janssen, Karyopharm, Merck, Regeneron, Takeda, Teneobio, Verastem; and research funding from Karyopharm. JPL has consulted for Sutro, Miltenyi, AstraZeneca, Epizyme, BMS/Celgene, Regeneron, Bayer, Gilead/Kite, Karyopharm, GenMab, Genentech/Roche, Abbvie, Incyte, Janssen.

Abstract

Currently available COVID-19 mRNA vaccines have demonstrated high efficacy in clinical trials.[1-3] However, cancer patients, including those with hematological malignancies, were largely excluded from these trials. In this prospective, observational study we measured anti-S protein IgG titers as well as avidity in lymphoma patients (n=67) vaccinated with a COVID-19 mRNA vaccine. Serological response rates in lymphoma patients who were treatment naïve (100% in CLL, 88.9% in other, non-CLL non-Hodgkin lymphoma patients), or who were last treated more than 24 months prior to vaccination (100% in CLL, 90% in other-NHL), were similar to healthy controls (100%). Patients on active therapy, however, had a diminished response rate (40% in CLL, 21.0% in other-NHL). No patient who received anti-CD20 monoclonal antibodies (mAb) within six months of vaccination responded. Thus, the utility of testing anti-S titers should be explored in patients on active therapy or with recent anti-CD20 mAb exposure, to assess their response to vaccination. We also propose studying passive protection with S-protein mAbs as an alternative prophylactic strategy for patients who respond poorly to vaccination.

Introduction:

The ongoing COVID-19 pandemic has affected over 172 million people globally with over 3.7 million deaths.[4] Three vaccines have received Emergency Use Authorizations (EUA) from the Food and Drug Administration (FDA) for use in the USA. Two mRNA-based vaccines encoding the SARS-CoV-2 spike protein (S), BNT162b2 (BioNtech/Pfizer) and mRNA-1273 (Moderna), have 95% and 94.1% efficacy against COVID-19, respectively.[1-3] Over 90% of study participants seroconvert by one week after the second dose of these vaccines, and remain antibody-positive for at least six months.[1-3,5,6] Both vaccines consistently elicit anti-S binding and neutralizing antibodies and T cell responses.[2,7]

The efficacy of SARS-CoV-2 vaccines in cancer patients may be more variable, particularly for individuals with hematologic malignancies or receiving immunosuppressive therapies. These patients may be at high risk for developing severe COVID-19 with poor outcomes.[8-11] To guide their care, it is important to understand how the approved COVID-19 vaccines perform in this patient population and identify subgroups that may not respond. Individuals receiving anti-CD20 monoclonal antibody (mAb) therapy, including lymphoma patients, are known to have relatively weak antibody responses to influenza vaccines.[12-15] A similar problem arises with influenza vaccination in CLL patients receiving the BTK inhibitor (BTKi), ibrutinib.[16,17] Similarly, only 3.8% of CLL patients taking a BTKi seroconverted to a hepatitis B vaccine, far lower than the 28% rate seen in the treatment-naïve patient group.[18] In turn, the latter seroconversion rate was much less than the >90% frequencies typically seen when hepatitis B vaccines are tested in healthy individuals.[19-21] Overall, there is now substantial evidence that B-cell targeted therapies, including anti-CD20 mAbs and BTKis, impede how vaccines perform.[16,17,22-24]

Very few cancer patients were included in the COVID-19 mRNA vaccine trials and any individuals receiving chemotherapy or immunotherapy within six months were excluded.[1,3] Consequently, we have an inadequate knowledge of how well these vaccines work in the

cancer patient population. However, by extrapolation from other vaccines, we hypothesized that patients with hematologic malignancies, especially those on immunosuppressive therapy, would produce poor serological responses to a COVID-19 vaccine.[25] We therefore measured anti-S protein ELISA titers induced by mRNA vaccines in lymphoma patients receiving various therapeutic interventions, especially B-cell targeted therapies. The avidity of antibody binding to the S-protein receptor binding domain (RBD) was also measured to determine if lymphoma or lymphoma therapies adversely affected the maturation of the antibody response. Finally, we determined whether any patients had serum antibodies to the SARS-CoV-2 nucleocapsid (N) protein, a marker for prior infection.[26] A past history of infection has been associated with a robust serological response to vaccination.[27-30]

Methods:

Study Design and Participants

In this single-center, observational cohort study we assessed antibody responses in lymphoma patients receiving a COVID-19 mRNA vaccine (BNT162b2 by BioNtech/Pfizer or mRNA-1273 by Moderna). All patients had provided written informed consent to participate in observational research, and this study was approved by the Weill Cornell Medicine IRB (IRB 21-02023288). Lymphoma patients, regardless of treatment status, underwent serum collection before and after vaccination. Whole blood samples used in this study were collected as part of standard of care laboratory testing protocols. Pre-vaccination samples were obtained at any time prior to the first vaccine dose, while post-vaccine samples were from at least 11 days after the second dose. When the mRNA vaccines became generally available, the study participants were strongly encouraged to receive a vaccine at the earliest opportunity and many did so. Thus, 22 patients had already received their first dose prior to sample collection. Pre- and post-vaccination samples were available from 22 of the 67 study participants, but only post-vaccination samples from the other 45.

We also include data from a healthy control group of 35 healthcare workers (HCWs) enrolled in the NYP-WELCOME (WEilL COrnell Medicine Employees) observational trial (IRB 20-04021831). The use of this cohort in an mRNA vaccine study has been previously described.[31]

Anti-S protein IgG ELISA

The assay to quantify IgG antibodies to the SARS-CoV-2 S-protein has been described previously.[31] Briefly, purified S-proteins (200 ng in 100 µl) were coated overnight onto 96-well plates at 4ºC. After 3 washes with PBS/0.05% Tween-20 (PBST), the wells were blocked for 1 h with 4% (w/vol) powdered milk/PBS (150 µl/well). Serum was initially diluted 1/100 in PBS containing 4% milk and 20% sheep serum, serially diluted as needed, and added to wells for 1 h. Bound antibodies were detected using goat anti-human IgG horse radish peroxidase (HRP)-conjugated antibodies from Jackson ImmunoResearch (109-035-008), diluted 1/5000 in 4% milk/PBS. After washing, 50 µl of HRP substrate (Thermo Scientific 34029) was added to each well for 3 min. Color development was terminated with 0.3 N sulfuric acid, and plates were read at 450 nm using an Enspire instrument (Perkin Elmer).

ELISA-derived net OD_{450} values were determined by subtracting background values from control wells containing no S-protein. Net OD_{450} values were plotted *vs.* $\log_{10}$ serum dilution before 3-parameter sigmoidal dose-response curves, constrained to zero, were fit (GraphPad Prism 9 software). The endpoint dilution cut-off was set to 0.100 net OD_{450} (i.e., 6 times the standard deviation of values from control wells with no S-protein). For inter-group comparison purposes, an endpoint titer value of 1:10,000 is marked on Figure 1A.

The Roche Elecsys Anti-SARS-CoV-2 N antigen assays

The Elecsys® Anti-SARS-CoV-2 electrochemiluminescence immunoassay was used to detect antibodies to the nucleocapsid (N) antigen in serum samples processed using the Roche Cobas e411 system (Roche Diagnostics, Indianapolis, IN). This assay has received EUA approval from the FDA and was used here to identify or confirm SARS-CoV-2 infection.

SARS-CoV-2 antibody avidity assay

The avidity assay measures the rate of antibody dissociation from the S-protein RBD, which is inversely correlated with antibody avidity. The assay is performed using the Pylon P3D analyzer (ET Healthcare, Palo Alto, CA) and has been described previously.[32] A probe coated with RBD proteins is sequentially exposed to diluted serum samples, a biotinylated-RBD protein and a Streptavidin-Cy5 conjugate to form a specific immunocomplex. *Signal_0* is the fluorescent signal before dissociation is measured. The probe and its immobilized immunocomplex then enters multiple repetitive dissociation cycles. *Signal_t* is the fluorescent signal measured at the end of each cycle, and directly correlates with the amount of antibody that remains probe-bound at the time of measurement. The *Signal_t* / *Signal_0* ratio at each time point is a measure of how much of the originally bound antibody has dissociated (i.e., [bound Ab] / [total Ab]). The dissociation profile is constructed by plotting the fluorescence signal ratio as a function of time. The relative dissociation rate (dR) is calculated by fitting the first order rate equation to the dissociation profile: Ln(Signal_t / Signal_0) = Ln([bound]/[total]) = -dR t.

Statistics

Data analysis and figures were produced in Prism 9 (GraphPad Software, La Jolla, Ca). Where indicated, p values to determine significance were calculated using unpaired, two-tailed t tests.

Results

The anti-S protein response to mRNA vaccination was assessed by ELISA using sera from 67 patients with lymphoma and 35 healthy HCW controls. The demographics and clinical characteristics of the lymphoma patients are listed in Table 1. The majority of patients in this study were white (74.6%). All patients were vaccinated with an mRNA vaccine (31 BNT162b2, 36 mRNA-1273). The patients were categorized as having Hodgkin lymphoma (n=4), chronic lymphocytic leukemia (n=21), or other non-Hodgkin lymphomas (n=42). No SARS-CoV-2 infections were identified during this study (February to April 2021).

Serum samples were obtained before (when possible) and after vaccination. Post-vaccination samples were collected within 11-70 days of the second dose (median 24.5 days). In the HCW control group, the post-vaccination samples were obtained within 10-68 days of dose-2 (median 40 days) (Figure S1).

Of the 22 lymphoma patients with samples available both pre- and post-vaccination, all but one were negative for S-protein antibodies prior to vaccination (Figure S2). That single S-protein antibody-positive patient was also positive for antibodies against the SARS-CoV-2 N-protein. This serology pattern implies that this patient had recovered from an undocumented, asymptomatic SARS-CoV-2 infection.

In total, 8 lymphoma patients were anti-N positive (including the individual referred to above); they are highlighted in blue in Figures 2-4. Notably, all members of the HCW control group were anti-N negative. As of now, no lymphoma patients have had a positive SARS-CoV-2 PCR test since the start of this study (i.e., when pre-vaccination sample collection commenced). For 4 of the 8 anti-N positive lymphoma patients, there was evidence of COVID-19 prior to the start of this study (Table S1). Thus, 3 patients had prior documented positive SARS-CoV-2 PCR tests, while the fourth was not PCR-tested but later had a positive commercial antibody test. Seven of these 8 anti-N positive patients responded to vaccination. Taken together, anti-N positive lymphoma patients had significantly higher mean anti-S protein titers than their anti-N negative counterparts ($p<0.0001$), and than the HCW group ($p=0.02$) (Figure S3). However, when anti-N positive lymphoma patients were separated by treatment status (i.e. naïve, active therapy) the sample sizes were too small for comparisons to the anti-N negative group.

The single anti-N positive patient who did not respond to vaccination is receiving active therapy with venetoclax and also receives a monthly IVIG infusion. IVIG is a possible alternative source of antibodies that react with the S- and N-proteins.[33-36] Hence it is possible that the positive N-protein serology seen in this patient arose from an IVIG infusion that was last given 3-weeks

before the date of post-vaccination sample collection. An alternative explanation is a previously undetected SARS-CoV-2 infection.

The vaccine-induced IgG antibody responses to the SARS-CoV-2 S-protein are shown in Figure 1A. The median and mean endpoint titers in the HCW control group were higher than in the lymphoma patients, although the difference was not significant. There were also no significant differences in mean titers when patients with different lymphomas were compared. However, while all 35 healthy control group members responded to the vaccine, a substantial proportion of the lymphoma patients did not. Thus, the anti-S endpoint titers in 9 of the 21 CLL patients and 17 of the 42 other NHL patients were <10,000 (a cut-off level marked on Figure 1A), and were often undetectable. In contrast, the 4 Hodgkin lymphoma patients all responded to the vaccines (Table S2). When the data were grouped according to whether the participants received the BNT162b2 or mRNA-1273 vaccine, no differences were apparent (Figure 1B).

We studied the CLL and other NHL patients in more detail to understand the implications of their treatment (Figure 2A). Every treatment-naïve and remote-therapy (no treatment in over 24 months) CLL patient responded to vaccination whereas only 40% (6/15) of those currently being treated had anti-S protein titers above the designated cut-off value (Figure 2A). Response rates in CLL patients receiving different therapy regimens are shown in Table S3.

A similar pattern was seen for the other NHL patients, although one individual in each of the treatment-naïve and remote-therapy groups failed to respond to the vaccine (Figure 2B). Active therapy in this sub-group was again associated with a poor vaccine response, with only 21.4% (3/14) developing anti-S protein titers above the cut-off (Figure 2B). Response rates in other NHL patients, grouped by therapy regimen, are listed in Table S4. The off-therapy sub-group, who had received treatment within 2 years but not at the time of vaccination, also had a lower vaccine response rate of 55.5% (5/9). The four non-responders in this group had all received an anti-CD20 mAb within the previous two years; two within 6 months, one within one year, and

one within 18 months (Table S5). None of the patients currently on anti-CD20 mAb therapy (1 CLL, 7 other NHL) seroconverted after vaccination (Fig. 2).

We next studied the relationship between when anti-CD20 mAb therapy ceased and the vaccine response (Figure 3). None of the 11 CLL and other NHL patients receiving this treatment within 6 months of vaccination had anti-S protein titers above the cut-off, but longer intervals were associated with higher titers. Thus, CLL and other NHL patients who were last treated >24 months before vaccination had response rates of 66.7% (6/9) and 71.4% (10/14), respectively. Of note is that 3/3 CLL and 3/4 other NHL non-responders in this sub-group were receiving a different type of active therapy at the time of vaccination (Table S6). We suggest that even when anti-CD20 mAb therapy ceased >24 months before vaccination, other forms of ongoing active therapy can compromise the vaccine response.

Finally, we studied the avidity of IgG antibodies to the RBD in the lymphoma and healthy control patients (Figure 4). The avidity was significantly higher ($p<0.0001$) for individuals in both the lymphoma and control groups who had anti-N antibodies (Figure 4A). That finding is expected, as COVID-19 convalescent patients (i.e., anti-N positive) have had longer to affinity mature their anti-S antibodies, which are boosted by the mRNA vaccines.[37-41] We noted that patients currently receiving venetoclax or a BTKi had lower avidity S-protein antibodies than the other groups, although the group sizes were too small for statistical significance (Figure 4B). If this preliminary observation is confirmed, an explanation might be reduced T-cell help for antibody maturation.

Discussion

We found that most lymphoma patients respond to vaccination with an mRNA-based COVID-19 vaccine, but a substantial fraction (>40%) do not and therefore may remain at risk of infection and disease. It is not yet known what constitutes a protective titer of S-protein antibodies after vaccination. Here, our primary endpoint was an ELISA that quantifies binding antibodies against

the S-protein, a subset of which will be capable of virus-neutralization. We consider an endpoint titer >1:10,000 in this assay to be a suitable indicator of a good response to vaccination.

Eight lymphoma patients were anti-N positive, which likely indicates prior SARS-CoV-2 infection (although one patient may have received N-protein antibodies via IVIG infusions). These patients had significantly higher anti-S IgG titers compared to anti-N negative lymphoma patients ($p<0.0001$) and HCW (all of whom were anti-N negative) ($p=0.02$). This result is consistent with reports that mRNA vaccines induce higher anti-S IgG titers in convalescent than naïve individuals.[27-30]

There were no significant differences in the S-protein IgG antibody response rates or titers between the different lymphoma histologic subtypes. Treatment status was, however, a relevant variable. Treatment-naïve lymphoma patients responded to vaccination similarly to the HCW group, as did patients who had not received therapy for at least two years.

There are reports of diminished mRNA vaccine responses in immunocompromised patients, including those receiving various lymphoma therapies.[12-18,25] For example, patients with chronic inflammatory diseases responded poorly compared to healthy controls, particularly those receiving glucocorticoids and B-cell depleting therapies.[42] In another study, nearly 20% of solid organ transplant patients were non-responsive to mRNA vaccination.[43,44] When 151 cancer patients received one dose of the BNT162b2 vaccine, only 38% of the solid cancer patients and 18% of those with hematologic malignancies (n = 56) seroconverted, compared to 95% of the healthy controls.[45] Among 67 patients with hematologic malignancies, including 34 lymphoma patients, nearly half did not respond to an mRNA vaccine.[46] Finally, when 44 CLL patients received an mRNA vaccine, only 23% of those on active therapy were positive for antibodies to the S-protein using a commercial assay.[47] These various reports are generally consistent with what we found. Specifically, commonly used lymphoma therapies can adversely influence the performance of COVID-19 vaccines, with anti-CD20 mAbs having the greatest impact.

To our knowledge, our study involves the largest cohort of non-CLL, non-Hodgkin lymphoma patients. In addition to assessing anti-S titers, our use of an avidity assay indicated that lymphoma patients who responded to the vaccines produced antibodies of comparable avidity to those found in the HCW group. Access to this healthy control group strengthens our conclusions about the lymphoma patients, as the vaccine response rate in the HCWs was similar to those seen in mRNA vaccine clinical trials.[1,3] Our study also offers a more detailed assessment of the temporal relationship between lymphoma treatment and vaccine response than has been the case to date, especially for anti-CD20 mAb therapy.

Among the cancer therapies monitored here, only the recent use of anti-CD20 antibodies was clearly associated with a poor response to the mRNA vaccines. In various studies, anti-CD20 mAb therapy causes B-cell depletion that is sustained for at least 6-12 months.[12,48-50] Given this knowledge, it is not surprising that 0% (0/11) of the patients who had received anti-CD20 mAb therapy within the prior 6 months had high-titer antibodies after vaccination. Better responses were seen as the time since anti-CD20 mAb infusion increased; after two years off therapy, 15 of 22 patients developed high-titer S-protein IgG antibodies (Figure 3). These observations have significant implications for patient care; the benefits of anti-CD20 therapy for CLL and other lymphomas should be balanced against the likely ablation of vaccine-mediated protection against COVID-19.

Recent studies suggest that using BTKi's in CLL patients also compromises SARS-CoV-2 vaccines.[16-18,22,24] Here, we found that 60% (6/10) of patients on BTKi monotherapy (4/6 in CLL, 2/4 in other NHL) did develop high-titer IgG antibodies after mRNA vaccination. There were some indications that antibody affinity was reduced compared to treatment naïve patients, perhaps reflecting reduced T-cell help, but our sample size was too small for us to draw strong conclusions. A larger cohort would be needed to further study the impact of this inhibitor class on vaccine-mediated protection.

The major weakness of this study is the sample size, which limited the statistical significance of observations made on subgroups of patients receiving specific therapies. Furthermore, many of our patients were on combination regimens (Tables S3-S4), which complicates analyses of the individual components. Also, we did not analyze T-cell responses to vaccination. Larger studies with longer follow-up, perhaps on a multi-center basis, would be required to address these lacunae. However, this controlled study presents compelling evidence that patients on active therapy for lymphoma may not respond to vaccination. Our results are particularly concerning for patients on anti-CD20 mAb therapy given that no patients who had received treatment within six months responded well to mRNA vaccination.

While we are not suggesting that COVID-19 vaccines are unsuitable for lymphoma patients, indeed any cancer patients, it would be prudent to assess whether they induce potentially protective S-protein antibodies, particularly when there are good grounds to believe the patient may be a non-responder. Another option may be to alter the timing of vaccination (i.e., before treatment begins) when possible. An attractive strategy may be an alternative way to protect against COVID-19: passive immunization with mAbs against the S-protein.[51] For example, REGEN-COV, a two-mAb combination (casirivimab and indevimab), has clinical efficacy as both a prophylactic and therapeutic agent. Preliminary Phase 3 trial results indicate that passive vaccination with REGEN-COV reduced the risk of symptomatic disease by 81% for household contacts of COVID-19 patients.[52] In another study, the same mAb combination also prevented symptomatic infection by 100% and PCR-detected infection by 50%, compared to placebo, with the infected participants in the mAb group having lower viral loads.[53] Similar results were reported for bamlanivimab, another anti-S mAb, in a non-peer reviewed press release.[54] In a Phase 3 clinical trial involving nursing home employees and residents, bamlanivimab reduced the risk of symptomatic infection by 80% compared to placebo.[54,55] When used as therapies, both REGN-COV and bamlanivimab reduce viral loads in COVID-19 patients.[56-58]

Passive immunization with anti-S mAbs for patients who do not respond to vaccination also has important public health implications. Several case reports have documented intra-host viral evolution within immunocompromised patients who have persistent SARS-CoV-2 infection, including ones with hematological malignancies.[59-63] Accordingly, there are concerns that new SARS-CoV-2 variants could arise under such circumstances.[64] Preventing infections with anti-S mAbs when active immunization is ineffective may reduce this risk to the community.

For various reasons, it is therefore appropriate to consider passive mAb protection for lymphoma patients who have recently (within 6-12 months) received anti-CD20 mAb therapy. Since anti-CD20 mAbs are commonly used in many other human diseases, the same COVID-19 protection strategy should also be considered more widely. While our study was not sufficiently powered to reach definitive conclusions about whether BTKi's and venetoclax reduce mRNA vaccine responses, there were indications of potential concerns that could be further evaluated in larger studies. A goal would, again, be to assess whether patients receiving these therapies may also benefit from passive mAb protection against COVID-19.

References and Notes

References:

1. Baden LR, El Sahly HM, Essink B, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. New England Journal of Medicine 2020;384:403-16.
2. Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. N Engl J Med 2020;383:1920-31.
3. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. New England Journal of Medicine 2020;383:2603-15.
4. WHO Coronavirus Disease (COVID-19) Dashboard. 2021.
5. Doria-Rose N, Suthar MS, Makowski M, et al. Antibody Persistence through 6 Months after the Second Dose of mRNA-1273 Vaccine for Covid-19. New England Journal of Medicine 2021.
6. Widge AT, Rouphael NG, Jackson LA, et al. Durability of Responses after SARS-CoV-2 mRNA-1273 Vaccination. New England Journal of Medicine 2020;384:80-2.
7. Sahin U, Muik A, Derhovanessian E, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature 2020;586:594-9.
8. Dai M, Liu D, Liu M, et al. Patients with Cancer Appear More Vulnerable to SARS-CoV-2: A Multicenter Study during the COVID-19 Outbreak. Cancer Discov 2020;10:783-91.
9. Miyashita H, Mikami T, Chopra N, et al. Do patients with cancer have a poorer prognosis of COVID-19? An experience in New York City. Ann Oncol 2020.

10. Mato AR, Roeker LE, Lamanna N, et al. Outcomes of COVID-19 in patients with CLL: a multicenter international experience. Blood 2020;136:1134-43.
11. Roeker LE, Scarfo L, Chatzikonstantinou T, et al. Worldwide Examination of Patients with CLL Hospitalized for COVID-19. Blood 2020;136:45-9.
12. Baker D, Roberts CAK, Pryce G, et al. COVID-19 vaccine-readiness for anti-CD20-depleting therapy in autoimmune diseases. Clin Exp Immunol 2020;202:149-61.
13. Kim W, Kim SH, Huh SY, et al. Reduced antibody formation after influenza vaccination in patients with neuromyelitis optica spectrum disorder treated with rituximab. Eur J Neurol 2013;20:975-80.
14. Yri OE, Torfoss D, Hungnes O, et al. Rituximab blocks protective serologic response to influenza A (H1N1) 2009 vaccination in lymphoma patients during or within 6 months after treatment. Blood 2011;118:6769-71.
15. Bar-Or A, Calkwood JC, Chognot C, et al. Effect of ocrelizumab on vaccine responses in patients with multiple sclerosis: The VELOCE study. Neurology 2020;95:e1999-e2008.
16. Douglas AP, Trubiano JA, Barr I, Leung V, Slavin MA, Tam CS. Ibrutinib may impair serological responses to influenza vaccination. Haematologica 2017;102:e397-e9.
17. Sun C, Gao J, Couzens L, et al. Seasonal Influenza Vaccination in Patients With Chronic Lymphocytic Leukemia Treated With Ibrutinib. JAMA Oncol 2016;2:1656-7.
18. Pleyer C, Ali MA, Cohen JI, et al. Effect of Bruton tyrosine kinase inhibitor on efficacy of adjuvanted recombinant hepatitis B and zoster vaccines. Blood 2021;137:185-9.
19. Halperin SA, Ward B, Cooper C, et al. Comparison of safety and immunogenicity of two doses of investigational hepatitis B virus surface antigen co-administered with an immunostimulatory phosphorothioate oligodeoxyribonucleotide and three doses of a licensed hepatitis B vaccine in healthy adults 18-55 years of age. Vaccine 2012;30:2556-63.
20. Heyward WL, Kyle M, Blumenau J, et al. Immunogenicity and safety of an investigational hepatitis B vaccine with a Toll-like receptor 9 agonist adjuvant (HBsAg-1018) compared to a licensed hepatitis B vaccine in healthy adults 40–70 years of age. Vaccine 2013;31:5300-5.
21. Jackson S, Lentino J, Kopp J, et al. Immunogenicity of a two-dose investigational hepatitis B vaccine, HBsAg-1018, using a toll-like receptor 9 agonist adjuvant compared with a licensed hepatitis B vaccine in adults. Vaccine 2018;36:668-74.
22. Abby PD, Jason AT, Ian B, Vivian L, Monica AS, Constantine ST. Ibrutinib may impair serological responses to influenza vaccination. Haematologica 2017;102:e397-e9.
23. Mauro FR, Giannarelli D, Galluzzo CM, et al. Response to the conjugate pneumococcal vaccine (PCV13) in patients with chronic lymphocytic leukemia (CLL). Leukemia 2021;35:737-46.
24. Zent CS, Brady MT, Delage C, et al. Short term results of vaccination with adjuvanted recombinant varicella zoster glycoprotein E during initial BTK inhibitor therapy for CLL or lymphoplasmacytic lymphoma. Leukemia 2020.
25. Shadman M, Ujjani C. Vaccinations in CLL: implications for COVID-19. Blood 2021;137:144-6.
26. Seow J, Graham C, Merrick B, et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. Nature Microbiology 2020;5:1598-607.
27. Abu Jabal K, Ben-Amram H, Beiruti K, et al. Impact of age, ethnicity, sex and prior infection status on immunogenicity following a single dose of the BNT162b2 mRNA COVID-19 vaccine: real-world evidence from healthcare workers, Israel, December 2020 to January 2021. Euro Surveill 2021;26.
28. Ebinger JE, Fert-Bober J, Printsev I, et al. Antibody responses to the BNT162b2 mRNA vaccine in individuals previously infected with SARS-CoV-2. Nature Medicine 2021.
29. Reynolds CJ, Pade C, Gibbons JM, et al. Prior SARS-CoV-2 infection rescues B and T cell responses to variants after first vaccine dose. Science 2021:eabh1282.

30. Goel RR, Apostolidis SA, Painter MM, et al. Distinct antibody and memory B cell responses in SARS-CoV-2 naïve and recovered individuals following mRNA vaccination. Science Immunology 2021;6:eabi6950.
31. Ketas TJ, Chaturbhuj D, Cruz-Portillo VM, et al. Antibody responses to SARS-CoV-2 mRNA vaccines are detectable in saliva. bioRxiv 2021.
32. Racine-Brzostek SE, Karbaschi M, Gaebler C, et al. TOP-Plus is a Versatile Biosensor Platform for Monitoring SARS-CoV-2 Antibody Durability. Clinical Chemistry 2021.
33. Díez JM, Romero C, Gajardo R. Currently available intravenous immunoglobulin contains antibodies reacting against severe acute respiratory syndrome coronavirus 2 antigens. Immunotherapy 2020;12:571-6.
34. Romero C, Díez JM, Gajardo R. Anti-SARS-CoV-2 antibodies in healthy donor plasma pools and IVIG products. Lancet Infect Dis 2021;21:765-6.
35. Farcet MR, Karbiener M, Schwaiger J, Ilk R, Kreil TR. Rapidly Increasing SARS-CoV-2 Neutralization by Intravenous Immunoglobulins Produced from Plasma Collected During the 2020 Pandemic. J Infect Dis 2021.
36. Ahn TS, Han B, Krogstad P, et al. Commercial immunoglobulin products contain cross-reactive but not neutralizing antibodies against SARS-CoV-2. J Allergy Clin Immunol 2021;147:876-7.
37. Victora GD, Nussenzweig MC. Germinal Centers. Annual Review of Immunology 2012;30:429-57.
38. Jerne NK. A study of avidity based on rabbit skin responses to diphtheria toxin-antitoxin mixtures. Acta pathologica et microbiologica Scandinavica 1951;87.
39. Eisen HN, Siskind GW. Variations in affinities of antibodies during the immune response. Biochemistry 1964;3:996-1008.
40. Ravichandran S, Coyle EM, Klenow L, et al. Antibody signature induced by SARS-CoV-2 spike protein immunogens in rabbits. Science Translational Medicine 2020;12:eabc3539.
41. Tang J, Ravichandran S, Lee Y, et al. Antibody affinity maturation and plasma IgA associate with clinical outcome in hospitalized COVID-19 patients. Nature Communications 2021;12:1221.
42. Deepak P, Kim W, Paley MA, et al. Glucocorticoids and B Cell Depleting Agents Substantially Impair Immunogenicity of mRNA Vaccines to SARS-CoV-2. medRxiv 2021.
43. Boyarsky BJ, Werbel WA, Avery RK, et al. Immunogenicity of a Single Dose of SARS-CoV-2 Messenger RNA Vaccine in Solid Organ Transplant Recipients. JAMA 2021;325:1784-6.
44. Boyarsky BJ, Werbel WA, Avery RK, et al. Antibody Response to 2-Dose SARS-CoV-2 mRNA Vaccine Series in Solid Organ Transplant Recipients. JAMA 2021.
45. Monin L, Laing AG, Muñoz-Ruiz M, et al. Safety and immunogenicity of one versus two doses of the COVID-19 vaccine BNT162b2 for patients with cancer: interim analysis of a prospective observational study. Lancet Oncol.
46. Agha M, Blake M, Chilleo C, Wells A, Haidar G. Suboptimal response to COVID-19 mRNA vaccines in hematologic malignancies patients. medRxiv 2021:2021.04.06.21254949.
47. Roeker LE, Knorr DA, Thompson MC, et al. COVID-19 vaccine efficacy in patients with chronic lymphocytic leukemia. Leukemia 2021.
48. Roll P, Palanichamy A, Kneitz C, Dorner T, Tony H-P. Regeneration of B cell subsets after transient B cell depletion using anti-CD20 antibodies in rheumatoid arthritis. Arthritis & Rheumatism 2006;54:2377-86.
49. Dörner T, Kinnman N, Tak PP. Targeting B cells in immune-mediated inflammatory disease: A comprehensive review of mechanisms of action and identification of biomarkers. Pharmacology & Therapeutics 2010;125:464-75.
50. Kneitz C, Wilhelm M, Tony HP. Effective B cell depletion with rituximab in the treatment of autoimmune diseases. Immunobiology 2002;206:519-27.
51. Taylor PC, Adams AC, Hufford MM, de la Torre I, Winthrop K, Gottlieb RL. Neutralizing monoclonal antibodies for treatment of COVID-19. Nature Reviews Immunology 2021.

52. Inc. RP. PHASE 3 PREVENTION TRIAL SHOWED 81% REDUCED RISK OF SYMPTOMATIC SARS-COV-2 INFECTIONS WITH SUBCUTANEOUS ADMINISTRATION OF REGEN-COV™ (CASIRIVIMAB WITH IMDEVIMAB). 2021.
53. Inc RP. REGENERON REPORTS POSITIVE INTERIM DATA WITH REGEN-COV™ ANTIBODY COCKTAIL USED AS PASSIVE VACCINE TO PREVENT COVID-19 . 2021.
54. Company ELa. Lilly's neutralizing antibody bamlanivimab (LY-CoV555) prevented COVID-19 at nursing homes in the BLAZE-2 trial, reducing risk by up to 80 percent for residents . 2021.
55. COVID-19 Antibody Treatment Study for Preventing SARS-CoV-2 Infection in Nursing Home Residents and Staff . 2021. at https://www.niaid.nih.gov/clinical-trials/covid-19-antibody-treatment-nursing-homes-blaze-2.)
56. Gottlieb RL, Nirula A, Chen P, et al. Effect of Bamlanivimab as Monotherapy or in Combination With Etesevimab on Viral Load in Patients With Mild to Moderate COVID-19: A Randomized Clinical Trial. JAMA 2021;325:632-44.
57. Weinreich DM, Sivapalasingam S, Norton T, et al. REGN-COV2, a Neutralizing Antibody Cocktail, in Outpatients with Covid-19. New England Journal of Medicine 2020;384:238-51.
58. Chen P, Nirula A, Heller B, et al. SARS-CoV-2 Neutralizing Antibody LY-CoV555 in Outpatients with Covid-19. New England Journal of Medicine 2020;384:229-37.
59. Baang JH, Smith C, Mirabelli C, et al. Prolonged Severe Acute Respiratory Syndrome Coronavirus 2 Replication in an Immunocompromised Patient. The Journal of Infectious Diseases 2020;223:23-7.
60. Reuken PA, Stallmach A, Pletz MW, et al. Severe clinical relapse in an immunocompromised host with persistent SARS-CoV-2 infection. Leukemia 2021;35:920-3.
61. Choi B, Choudhary MC, Regan J, et al. Persistence and Evolution of SARS-CoV-2 in an Immunocompromised Host. New England Journal of Medicine 2020;383:2291-3.
62. Hensley MK, Bain WG, Jacobs J, et al. Intractable Coronavirus Disease 2019 (COVID-19) and Prolonged Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Replication in a Chimeric Antigen Receptor-Modified T-Cell Therapy Recipient: A Case Study. Clinical Infectious Diseases 2021.
63. Kemp SA, Collier DA, Datir RP, et al. SARS-CoV-2 evolution during treatment of chronic infection. Nature 2021.
64. Abbasi J. Researchers Tie Severe Immunosuppression to Chronic COVID-19 and Virus Variants. JAMA 2021;325:2033-5.

FIGURES AND TABLES

FIGURES AND TABLES

Characteristics		
Sex		
	Male	36
	Female	31
Mean Age		68.8
Median Age		71 (24-90)
Ethnicity		
	Hispanic or Latino	0
	White	50
	Black	1
	Asian	1
	American Indian or Alaska Native	0
	Native Hawaiian or Other Pacific Islander	1
	Multiracial	4
	Not reported or unknown	10
Vaccine		
	BNT162b2 (BioNTech/Pfizer)	31
	mRNA-1273 (Moderna)	36
Comorbidities		
	Hypertension	25
	Chronic lung disease	1
	Liver Disease	0
	Heart Disease	17
	Kidney Disease	5
	Autoimmune Disease	8
	Diabetes	4
	HIV	0
	Other Cancer	21
	Hyperlipidemia	34
Mean BMI		26.59 (±4.49)
Malignancy		
	HL	4
	CLL	21
	other NHL	42
	FL	7
	MZL	10
	MCL	8
	DLBCL	8
	WM	7
	Other	2

Table 1: Baseline characteristics of lymphoma patients (n=67).

(HL = Hodgkin Lymphoma, CLL = chronic lymphocytic leukemia, NHL = non-Hodgkin

lymphoma, FL = follicular lymphoma, MZL = marginal zone lymphoma, MCL = mantle cell lymphoma, DLBCL = diffuse large B-cell lymphoma, WM = Waldenstrom's macroglobulinemia)

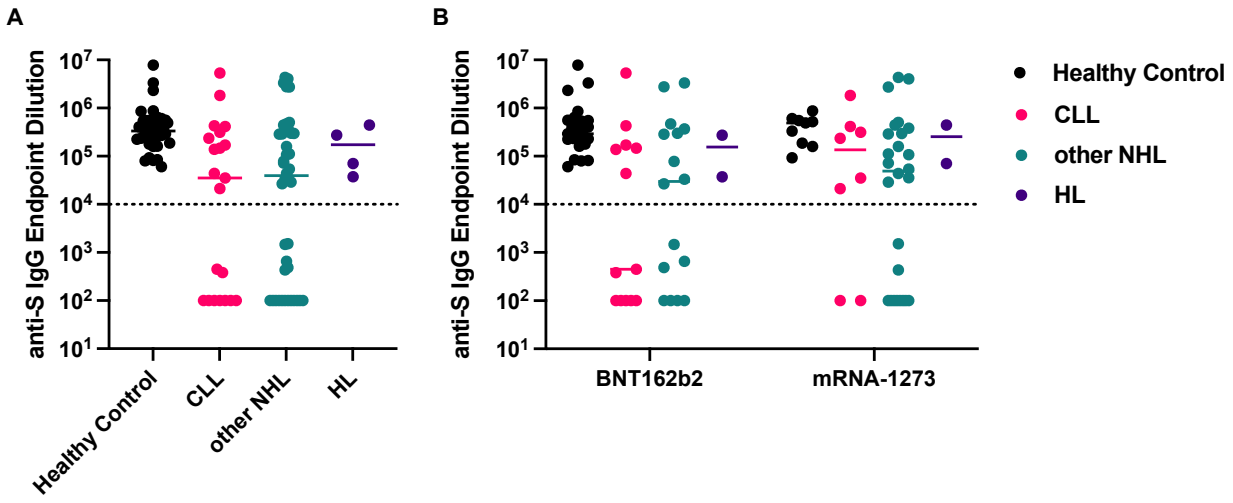

Figure 1: Anti-S IgG titers for healthy control and lymphoma patients (A) SARS-CoV-2 S-protein antibody (anti-S IgG) endpoint ELISA titers for healthy control (n=35), chronic lymphocytic leukemia (n=21), and other non-Hodgkin lymphoma (n=42) patients. Blood samples were collected at least 11 days following inoculation with the second dose of an mRNA vaccine. The dotted line represents an endpoint anti-S protein titer (1:10,000) that we judge to be an indicator of a strong response to vaccination. The small solid lines indicated the median titers for each group. There were no significant differences between the groups (unpaired, two-tailed t-tests). (B) Anti-S IgG titers for the indicated study groups, categorized by the mRNA vaccine used: BNT162b2 (Pfizer) or mRNA-1273 (Moderna).

Figure 2

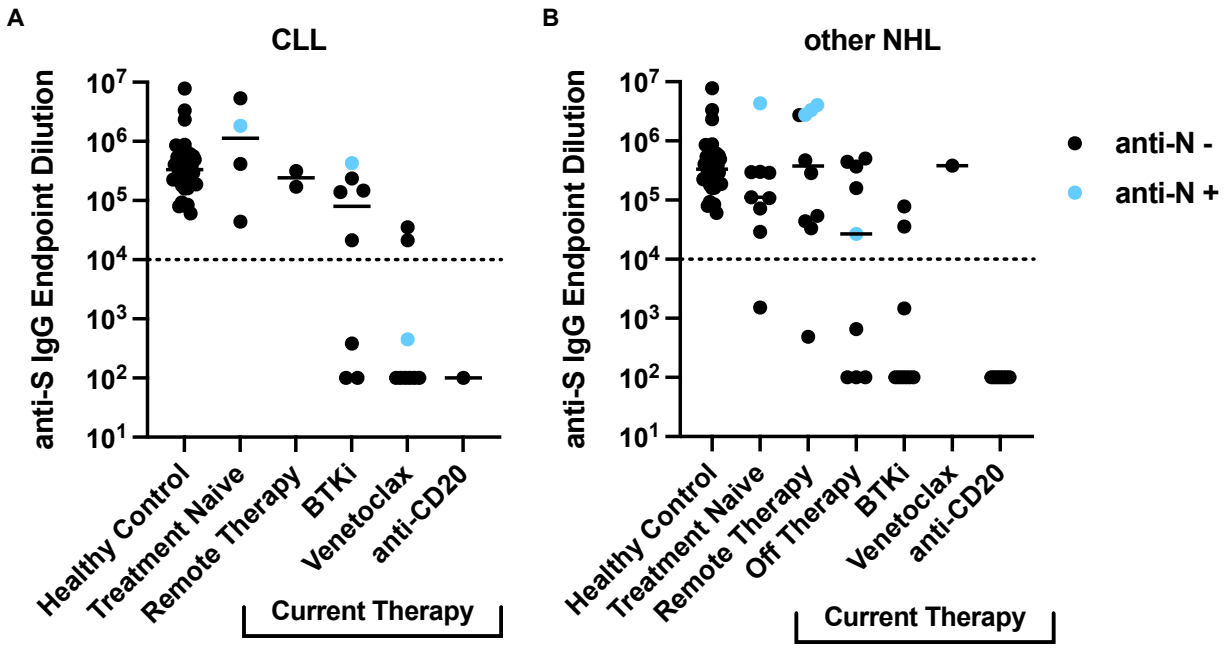

Figure 2: Anti-S IgG titers by therapy (A) Anti-S IgG for CLL patients separated by treatment status and for the healthy control group. The treatment-naïve patients (n=4) received no therapy at any time. The remote-therapy patients (n=2) received no treatment within the 24 months prior to vaccination. The off-therapy patients received treatment within 24 months of vaccination but not during or after (this sub-group does not no overlap with either the remote- or current-therapy groups, and applies only to the other NHL patients). Patients currently receiving therapy were treated as indicated: Bruton's tyrosine kinase inhibitor (BTKi; n=7), venetoclax (n=9), anti-CD20 therapy (n=1). The blue symbols indicate patients (n=3) who were positive for anti-N antibodies, which most likely arise from prior SARS-CoV-2 infection (see main text). (B) As for panel-A, but for Other NHL patients. Treatment-naïve (n=9); remote-therapy (n=10); off-therapy (n=9); current therapies including BTKi (n=6), venetoclax (n=1), anti-CD20 (n=6). Anti-N positive patients (n=5) are again in blue.

Figure 3

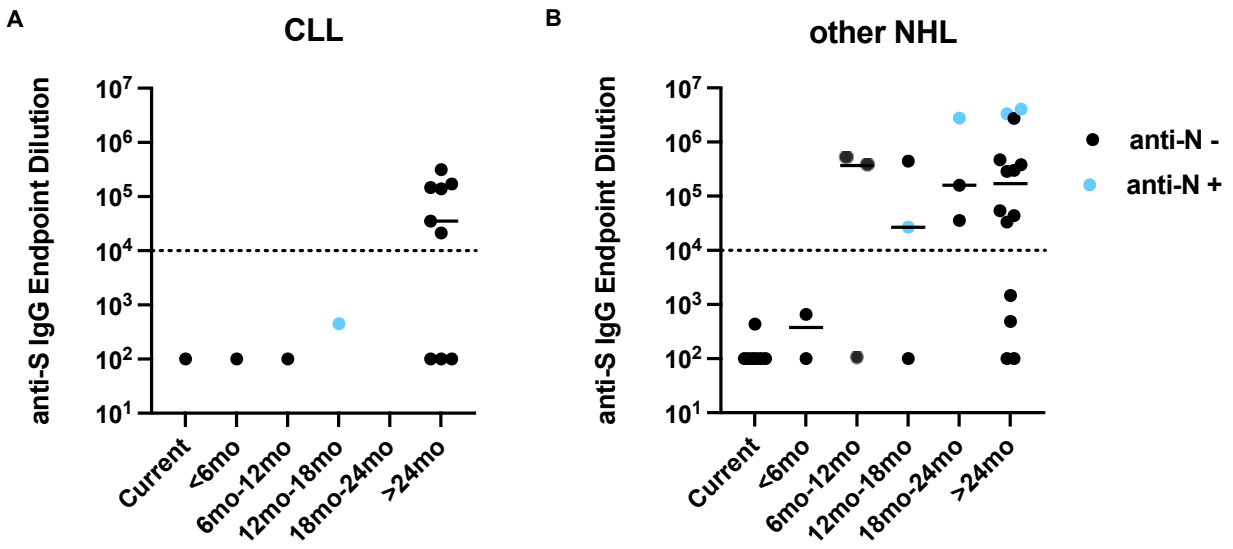

Figure 3: Anti-S IgG titers according to last anti-CD20 therapy (A) Anti-S IgG titers for CLL patients grouped by the time interval since anti-CD20 therapy ended. The Current group was receiving anti-CD20 at the time of vaccination (n=1). The other groups are designated according to how long therapy ceased before vaccination: <6mo (n=1); 12-18 months (n=1); 18-24 months (n=1); > 24 months (n=8). Anti-N positive patients are highlighted in blue. (B) As for panel-A but for other NHL patients: Current (n=7); <6 months (n=2); 6-12 months (n=3); 12-18 months (n=3); 18-24 months (n=3), >24 months (n=17).

Figure 4

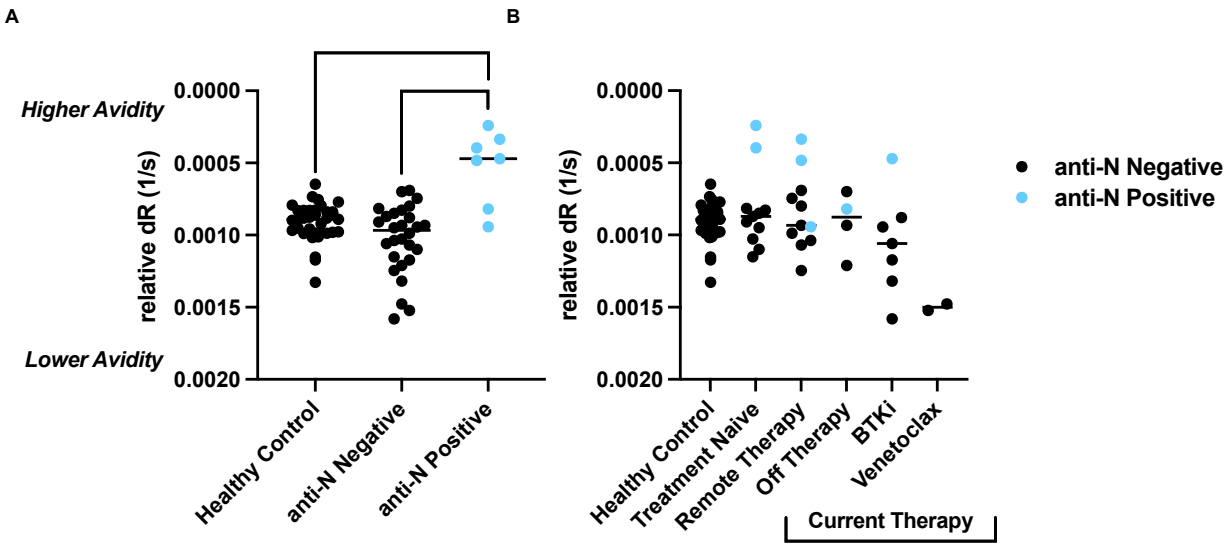

Figure 4: anti-S total antibody avidity (A) Avidity of healthy control (n=35), anti-N negative lymphoma patients (n=28) and anti-N positive lymphoma patients (n=7). A low relative dissociation rate (relative dR, y-axis) corresponds to high avidity and as such the y-axis is inverted. There were significant differences between the indicated (****) groups ($p<0.0001$),as determined using an unpaired, two tailed t-test. (B) As for panel-A but with CLL and other NHL patients grouped by therapy type: treatment-naïve (n=11); remote-therapy (n=11); off-therapy (n=4); BTKi (n=7); venetoclax (n=2). Avidity in patients on venetoclax was significantly decreased compared to treatment naïve ($p=0.0073$), remote therapy ($p=0.0063$), and off therapy ($p=0.0238$) groups. There were no significant differences between other treatment groups.

SUPPLEMENTARY TABLES AND FIGURES

Patient	Lymphoma	Pre anti-S IgG	Post anti-S IgG	Positive SARS-CoV-2 PCR	Commercial Serology	Clinical History	IVIG
24	WM	N/A	4349034	No	Positive (4/2020)	Yes (03/2020)	No
27	DLBCL	N/A	3338448	No	No	No	No
64	MCL	N/A	4070709	No	No	No	No
23	CLL	N/A	426856	Yes (04/2020)	Positive (07/2020)	Yes (hospitalized 04/2020)	No
44	FL	1657	26870	No	No	No	No
32	CLL	N/A	1835830	Yes (03/2020)	No	Yes (03/2020)	No
29	DLBCL	N/A	2759055	Yes (06/2020)	No	Yes (06/2020)	No
7	CLL	N/A	451	No	Negative (02/09/2021)	No	Monthly

Table S1: Anti-N positive patients listed according to lymphoma subtype and history of SARS-CoV-2 infection. Note that the commercial serology test for these patients detects antibodies to the SARS-CoV-2 N protein. Clinical history refers to documented symptoms consistent with COVID-19. Patient 7 last received IVIG, 21 days prior to their post-vaccination sample collection.

(DLBCL = diffuse large B-cell lymphoma, MCL = mantle cell lymphoma, FL = follicular lymphoma)

	Response	Mean anti-S IgG
Healthy Control	35/35 (100%)	707491
CLL	12/21 (57.14%)	434858
other NHL	25/42 (59.52%)	508316
HL	4/4 (100%)	206207

Table S2: Response rate and mean anti-S IgG tiers for healthy controls and lymphoma patients

	Response	anti-S IgG
Treatment Naïve	4/4 (100%)	1910213
Remote Therapy	2/2 (100%)	241468
Off Therapy	N/A	N/A
Active Therapy	6/15 (40%)	67216
BTKi	4/6 (66.67%)	166062
Venetoclax	1/4 (25%)	9018
BTKi+Venetoclax	1/2 (50%)	10716
PI3Ki + Venetoclax	0/2 (0%)	100
PI3Ki + Venetoclax + anti-CD20 mAb	0/1 (0%)	100
Total	12/21 (57.14%)	

Table S3: Vaccine response in CLL patients according to therapy. Note that no CLL patients were considered “off therapy.”

(BTKi = Bruton’s tyrosine kinase inhibitor, PI3Ki = PI3 kinase inhibitor)

	Response	anti-S IgG
Treatment Naïve	8/9 (88.89%)	1112801
Remote Therapy	9/10 (90%)	819119
Off Therapy	5/9 (55.55%)	117310
Active Therapy	3/14 (21.43%)	35767
BTKi	2/4 (50%)	28880
Venetoclax	1/1 (100%)	383979
anti-CD20 mAb	0/1 (0%)	100
PI3Ki + BTKi	0/1 (0%)	100
Bendamustine + BTKi	0/1 (0%)	100
Bendamustine + anti-CD20 mAb	0/1 (0%)	100
Lenalidomide + anti-CD20 mAb	0/1 (0%)	100
BTKi + Lenalidomide + anti-CD20 mAb	0/3 (0%)	211
Total	25/42 (59.52%)	

Table S4: Vaccine response in non-NHL patients according to therapy

(BTKi = Bruton's tyrosine kinase inhibitor, PI3Ki = PI3 kinase inhibitor)

Patient	Lymphoma	Pre IgG	Post IgG	Last Therapy	Last anti-CD20 mAb (days)
66	MZL	N/A	100	Rituximab	156
47	DLBCL	N/A	100	R-CHOP	469
53	DLBCL	N/A	100	R-CHOP	270
61	DLBCL	100	655	R-CHOP	165

Table S5: Off-therapy other-NHL patients who did not respond to vaccination. All of these patients were last treated with an anti-CD20 mAb as part of their most recent therapy. Each patient is listed with lymphoma subtype and most recent therapy (R-CHOP = rituximab + cyclophosphamide, doxorubicin, vincristine, and prednisolone). Also shown is the time (in days) between date of last anti-CD20 mAb therapy and the date of second vaccine dose. (DLBCL = diffuse large B-cell lymphoma, MZL = marginal zone lymphoma)

Patient	Lymphoma	Pre anti-S IgG	Post anti-S IgG	Current Therapy
13	CLL	N/A	100	Ibrutinib+venetoclax+IVIG
17	CLL	100	101	Venetoclax
41	CLL	N/A	100	Venetoclax+IVIG
22	MZL	N/A	100	Bendamustine+Ibrutinib
25	MCL	N/A	100	Zanubrutinib
34	MZL	N/A	1476	Zanubrutinib+Plasmapharesis
56	MZL	100	487	None

Table S6: Patients treated with anti-CD20 mAb over 24 months prior to vaccination

Ibrutinib and zanubrutinib are BTKi's. (MZL = marginal zone lymphoma, MCL = mantle cell lymphoma)

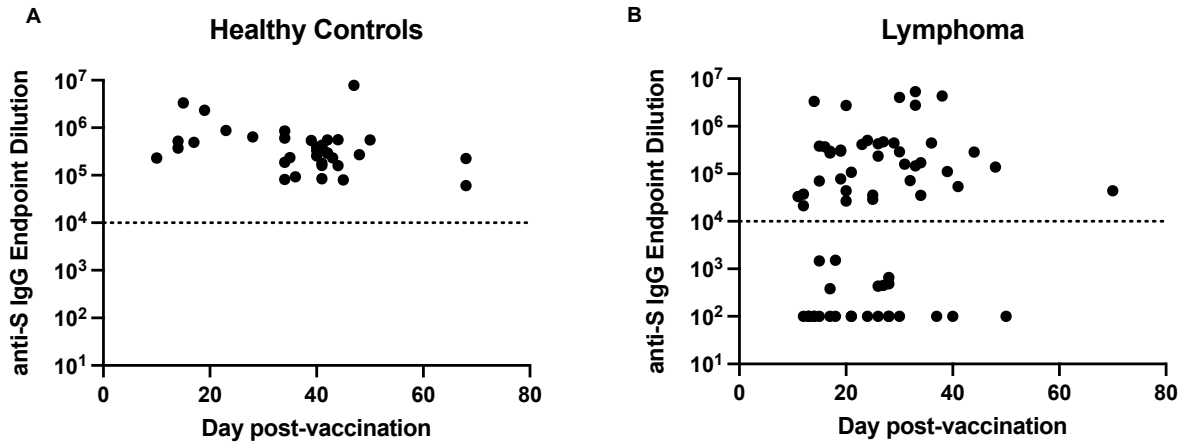

Figure S1: Anti-S IgG Titers by sample collection day post-vaccination (A) Anti-S IgG in healthy control patients plotted against the time of sampling post-vaccination. (B) As for panel-A but for lymphoma patients.

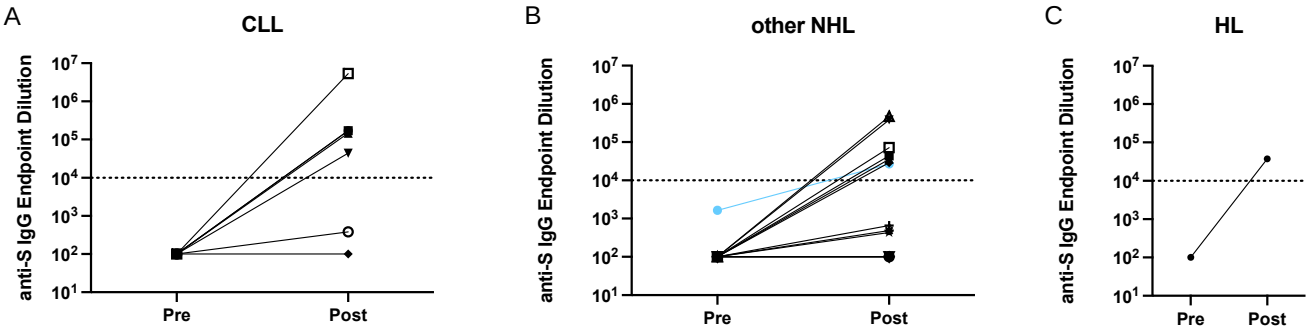

Figure S2: Pre and post vaccination titers by lymphoma subtype (A) Anti-S IgG titers for CLL patients (n=7). Each symbol represents a single patient, with the line connecting the pre- and post-vaccination titers. (B) As for panel-A but for other NHL patients (n=14). Note that the one patient with a quantifiable (although low) anti-S titer pre-vaccination titer was also anti-N positive, suggestive of a prior SARS-CoV-2 infection. (C) As for panel-A but for a HL patient (n=1)

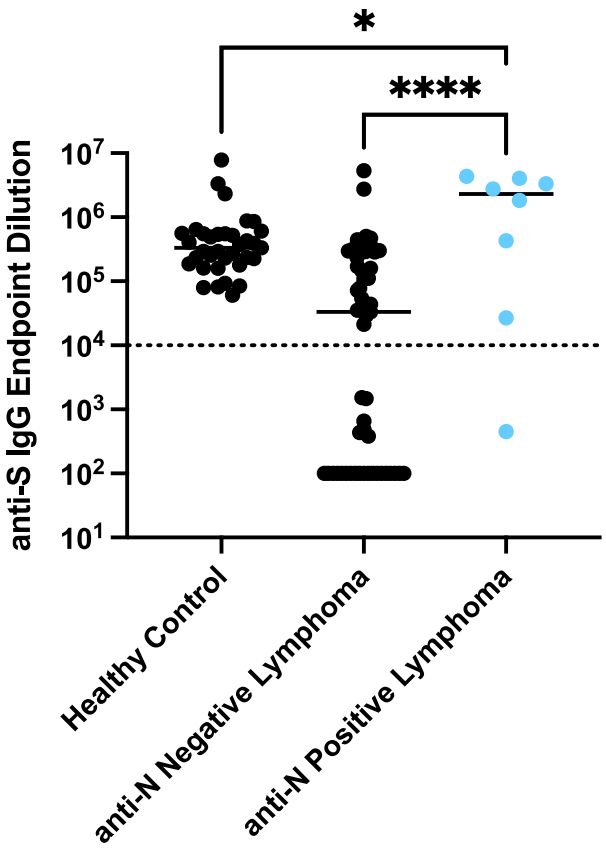

Figure S3: anti-S IgG titers by prior infection. Anti-N positive lymphoma patients had significantly higher anti-S IgG titers compared to anti-N negative lymphoma patients (****, p<0.0001) and anti-N negative HCW (*, p=0.02) as determined by unpaired, two-sided t tests. The difference between anti-N negative lymphoma patients and anti-N negative HCW was not significant (p=0.05). Note that the one patient in the anti-N positive group who fell below the titer threshold for a meaningful response (1:10000) was receiving IVIG.